Lincoln University
Te Whare Wānaka o Aoraki
CHRISTCHURCH•NEW ZEALAND

Lincoln University Digital Thesis

Copyright Statement

The digital copy of this thesis is protected by the Copyright Act 1994 (New Zealand).

This thesis may be consulted by you, provided you comply with the provisions of the Act and the following conditions of use:

- you will use the copy only for the purposes of research or private study
- you will recognise the author's right to be identified as the author of the thesis and due acknowledgement will be made to the author where appropriate
- you will obtain the author's permission before publishing any material from the thesis.

Effects of nitrogen fertiliser and regrowth interval on herbage dry matter yield, chemical composition and nitrogen solubility of alternative pasture forages in irrigated Canterbury conditions

---

A thesis

submitted in partial fulfilment

of the requirements for the Degree of

Doctor of Philosophy

at

Lincoln University

by

Kirsty Eve Martin

Lincoln University

2018

Abstract of a thesis submitted in partial fulfilment of the requirements for the Degree of Doctor of philosophy.

Effects of nitrogen fertiliser and regrowth interval on herbage dry matter yield, chemical composition and nitrogen solubility of alternate pasture forages under irrigated Canterbury conditions

by

Kirsty Eve Martin

The New Zealand agricultural sector is challenged with finding approaches to reduce the loss of nitrogen (N) through nitrate leaching, without the loss of farm production and profitabilty. One way to do this is to use different forages, regrowth intervals and N fertiliser rates to manipulate herbage N concentration and plant nitrogen use efficiency (NUE). The objective of this thesis was to quantify, for a range of pasture forages, herbage dry matter (DM) yield, chemical composition, N solubility and herbage N fertiliser requirements in response to different rates of N fertiliser, and regrowth interval in irrigated pastures in Canterbury.

The thesis reports on four experiments. The objective of experiment 1 was to quantify the effect of N fertiliser rate on herbage DM yield and chemical composition, of two herb species (plantain and chicory) three legume species (lucerne, red clover and white clover) and seven grass species (prairie grass, Italian ryegrass, tall fescue, tetraploid perennial ryegrass, diploid perennial ryegrass, high sugar perennial ryegrass and cocksfoot) at optimum defoliation time over a two year period. In this study, conducted on the Canterbury Plains, New Zealand, the twelve species were grown at six N fertiliser rates ranging from 0 to 450 kg N/ha/year and managed under irrigation and cutting management. Herbage DM yield and N concentration were measured at each harvest over two years (1 December 2014 to 30 November 2016). Defoliation management was on average, every 29 days in grasses and herbs with a total of 9 harvests per year, and 38 days in legumes with a total of 7 harvests per year. Forages were not harvested over the winter (June, July). As N fertiliser rate increased from 0 to 450 kg N/ha/year, annual herbage production increased linearly, from 5016 to 13812 kg DM/ha/year in grasses and from 6844 to 11481 kg DM/ha/year in herbs. In contrast, annual DM yield for legumes was unaffected by N fertiliser rate and ranged from 10596 to 10936 kg DM/ha. Additionally, there were contrasting ($P<0.001$) responses in herbage N concentration between species. At all N fertiliser rates,

annual herbage N concentration was highest in legumes (4.3 % N), intermediate in herbs (3.1 % N), and lowest in grasses (2.7 % N). The herbage N concentration of legumes were unaffected by increasing N fertiliser rate (4.4 % N at 0 kg N/ha/yr to 4.5 % N at 450 kg N/ha/yr), whereas in grasses and herbs it increased. However, the increase in N concentration in the grasses and herbs as N fertiliser rate increased, was small (2.6 % N at 0 kg N/ha/yr to 3.2 % N at 450 kg N/ha/yr, $P<0.001$). Thus, it is concluded for plantain, chicory, prairie grass, Italian ryegrass, tall fescue, tetraploid perennial ryegrass, diploid perennial ryegrass, high sugar perennial ryegrass and cocksfoot, the reduction in use of N fertiliser as a mitigation strategy to decrease herbage N intake may be ineffective. In addition, the results suggest that there were no benefits in using herbs instead of grasses for reducing N intake in livestock in an irrigated Canterbury environment.

The objective of experiment 2, was to quantify the effect of N fertiliser rate on herbage DM yield and chemical composition of plantain, chicory, red clover, white clover, diploid perennial ryegrass and cocksfoot at 4 regrowth intervals (1, 2, 3 and 4 weeks) in spring and autumn. In this study, conducted on the Canterbury Plains, New Zealand, the six species were grown at three N fertiliser treatments; nil (0 kg N/ha/year), medium (180 kg N/ha/year) and high (450 kg N/ha/year), and managed under irrigation and cutting management. The effect of regrowth interval and fertiliser rate on herbage DM yield, plant N concentration, water soluble carbohydrate: crude protein (WSC: CP) ratio and digestible organic matter in the DM (DOMD) were measured each week over a 4-week regrowth period, in autumn 2015 and spring 2016. As regrowth interval increased, herbage DM yield increased (from 179 kg DM/ha to 922 kg DM/ha in the autumn, and from 169 kg DM/ha to 1772 kg DM/ha in the spring, $P<0.05$). Averaged over the N fertiliser rates, herbage DM yield was greatest in plantain (1544 kg DM/ha/yr) and lowest in diploid perennial ryegrass (1148 kg DM/ha/yr). N response rates were highest in perennial ryegrass (31.5 kg DM/kg N applied) and plantain (32.2 kg DM/kg N applied, $P<0.05$). Herbage N concentration of grasses and herbs in autumn was high (>3.2% N, averaged over the N fertiliser rates), compared to spring (2.5 % N, averaged over the N fertiliser rates) and increased with N fertiliser rate (3.1 N % at nil N fertiliser to 3.9 N % at high N fertiliser, $P<0.001$), but decreased with regrowth interval (3.8 N % at week 1 to 2.9 N % at week 4, $P<0.001$). The difference in herbage N concentration between N fertiliser rates was notably higher at the beginning of the regrowth interval, compared to after 4 weeks of regrowth (12.4 % difference between nil and high N fertiliser at 1-week regrowth, and 10.2 % difference between nil and high N fertiliser). Thus, manipulating regrowth interval may reduce herbage N intake more so than N fertiliser application. Overall, delaying grazing to 4 weeks, under a moderate N regime, did not reduce herbage quality but reduced herbage N concentration in autumn and spring. These results suggest plantain is a suitable alternative to perennial ryegrass to reduce N losses without impeding farm production because of its high N response rates, high herbage DM yield and low herbage N concentrations, compared to the other species.

The objective of experiment 3 was to determine the herbage N concentration and N fertiliser requirements in diploid perennial ryegrass, high sugar perennial ryegrass grass, Italian ryegrass, cocksfoot, prairie grass, chicory and plantain at maximum herbage DM yield under glasshouse conditions over a six-month summer and autumn period. In this study, the seven forages were grown over 19 weeks under seven N fertiliser rates, ranging from 0 – 40 g N/m^2 (0 – 400 kg DM/ha) total N applied. Accumulated herbage DM yield was highest and N concentration lowest in prairie grass (456 g DM/m, 1.9 % N) and plantain (451 g DM/m^2, 1.9 % N), averaged over the N fertiliser rates. At the highest (40 g N/m^2) N fertiliser rate, response rates were lower (20.9 g DM/g N applied) than the low (17.5 g N/m^2) N fertiliser rates (27.4 g DM/g N applied). This suggests that the utilisation of N for higher herbage DM yields was not as effective when high amounts of N fertiliser was applied. This was shown in the soil N concentration (soil mineralisable N) which was highest at the high N fertiliser rate (8.6 g/m^2 at the 40 g N/m^2 N fertiliser rate vs 7.0 g N/m^2 at the 17.5 g N/m^2 N fertiliser rate). Herbage N concentration increased when applications of 10 g N/m^2 or above were applied to forages, although large differences were not shown until the highest N rate was applied (2.0 % N vs 2.5% N). This result indicates the difficulty in altering herbage N concentration in plants at the final harvest, as found in the previous chapters. Overall, the high herbage DM yields and low N concentrations in prairie grass and plantain found in this trial indicates they could be used to reduce herbage N intake without impeding on farm production.

The objective of experiment 4 was to quantify the effect on N fertiliser rate on chemical composition and N solubility in plantain, chicory, lucerne, white clover, diploid perennial ryegrass and cocksfoot at optimum defoliation time in the summer and autumn. This study examined the six forages under two N fertiliser rates (180 kg N/ha/year and 450 kg N/ha/year) using herbage collected in summer and autumn. Grasses were the highest ($P<0.01$) in neutral detergent insoluble N (NDIN) (380 mg/g N) and lowest in non-protein N (NPN) (111 mg/g N). Herbs contained higher ($P<0.0.1$) amounts of insoluble N (836 mg/g N) that was slower to degrade in the rumen compared to legumes (766 mg/g N) and grasses (511 mg/g N). Legumes were shown to be higher in NPN (180 mg/g N), than grasses (111 mg/g N) and herbs (76 mg/g N) which is easily converted to urea and urinary N. As a result, legumes may contribute to higher urinary losses in pastoral systems. The addition of high N fertiliser applications increased NPN in all species from 97 mg/g N to 148 mg/g N. Results from this trial suggest herbs contain N that is more available to be utilised by the animal for growth and production, reducing the amount of N lost as urinary N. In addition, high N fertiliser rates may lead to higher N intakes.

Overall, the use of Italian ryegrass, prairie grass, tall fescue, perennial ryegrass, high sugar ryegrass and plantain, with lower N fertiliser rates and 4-week regrowth intervals could be used to reduce herbage N intake. However, the use of legumes in traditional ryegrass-white clover pastures may contribute to higher N losses in the system. Moreover, the herbage DM yield, N concentration and N solubility results

from this PhD have indicated the use of alternative pasture forages may be incorporated into farm systems without hindering farm production or profitability.

Keywords: Plantain, chicory, perennial ryegrass, cocksfoot, prairie grass, tall fescue, high sugar perennial ryegrass, Italian ryegrass, lucerene, red clover, white clover, nitrate leaching, nitrogen response rates, water soluble carbohydrates, nitrogen solubilty, nitrogen use efficiency.

Acknowledgements

I firstly wish to thank my supervisors Professor Grant Edwards, Dr Racheal Bryant and Associate Professor Jim Moir for their guidance, support and encouragement during my time carrying out this PhD. I would also like to thank Dr Mirian Hodge and Dr Simon Hodge for their help with my statisical analysis.

I would like to acknowledge the *Forages for Reduced Nitrate Leaching* programme, with principal funding from the New Zealand Ministry of Business, Innovation and Employment. The programme is a partnership between DairyNZ, AgResearch and Landcare Research.

A very special thanks goes to my fellow PhD peers under the *Forages for Reduced Nitrate Leaching* programme Lisa Box, Grace Cun, Anna Carlton and Rosheen Woods.

I would like to thank Lincoln University Research Dairy Farm staff; Jeff Curtis, Helen Hague, Misato Iitaka, Caleb Sixtus and Sarah Taylor who help me with my exerpimental work in the paddock. Also, thank you to the Field Research Centre staff; Dave Jack, Dan Dash and Malcolm Smith who were always a breeze to work with and kept me company in between endless hours of grinding samples. Finally, to the ladies in the Riddols Building; Shuang Jiang, Rosy Tung, Jenny Zhao and Vicky Jiao Zhang, thank you for your knowledge, help and good company in the laboritory.

Thank you also to my colleagues Rhianon Handcock, Lucy Burrows, Aimi Nabilah Hussein, Ao Cheng, Dr Frisco Nobilly, Dr Tom Maxwell, Dr Omar Al-Marashdeh, Dr Paul Long Cheng and Dr Laura Rossi for your friendship and support during my PhD process.

Finally I would like to thank my partner Will Thomas for the encouragement, support and reassurance as I took on this mamouth task and to my family Andrew and Racheal Martin, Emily Verity and Peter.

Project Information

This project was completed as part of the Forages for Reduced Nitrate Leaching Programme with principal funding from the Ministry of Business, Inovation and Employment, and DairyNZ. The programme was a partnership between DairyNZ, AgResearch, Plant and Food Research, Lincoln University, Foundation of Arable Research, and Landcare Research.

Some of the results from this thesis have been presented at the following conferences and submitted for pubilication:

- Martin, K.E., Edwards, G.R., Bryant, R., Hodge, M., Moir, J., Chapman, D., Cameron, K.C., (2017) Herbage dry-matter yield and nitrogen concentration of grass, legume and herb species grown at different nitrogen-fertiliser rates under irrigation. Animal Production Science 57, 1283-1288.

- Martin, K.E., Bryant, R., Hodge, S., Edwards, G.R., (2017). Effect of autumn regrowth interval and nitrogen fertiliser on dry matter yield and plant characteristics of six forage species. Conference: New Zealand Grassland Association Whanganui, Volume: 79: 67-72

- Woods, R.R., Carlton, A.J., Martin, K.E., Box. L, Cun, G., (2017). Practical options for reducing the environmental impacts of intensive forage-based dairy systems. South Island Dairy Event Controlling the Controllable, 26 – 28 June 2017, Lincoln, New Zealand.

Table of Contents

List of Tables

List of Figures

List of Plates

Chapter 1

Introduction

1.1 Introduction

The New Zealand agricultural sector in the last 20 years had gone through dramatic intensification. Approximately 28% of New Zealand's 2016 total export revenue was attributed to agriculture, which included milk products, meat products, cereals and wool (Statistics New Zealand 2017). The rapid expansion of agriculture, especially the dairy sector, has been in part to the development of irrigation. This has allowed areas that were originally dryland to convert into large scale dairy or cropping systems, especially in Canterbury and North Otago. However, this has caused environmental concerns around the level of nitrate leaching into waterways particularly through the production of urine by dairy cows and the inefficient use of nitrogen (N) fertiliser (Valentine and Kemp 1999). To combat the water quality issue, a National Policy Statement for Freshwater Management (NPSFM) was created and came into effect in New Zealand in 2011 (Ministry for the Environment 2014). Under these rules, regional councils must manage and set water quality limits. In turn, such limits could affect typical farm practices through need to achieve lower leaching loss targets. Therefore, an important goal for New Zealand farm systems is to maintain or increase profitability while achieving environmental targets associated with lower nitrate leaching from soils.

One approach to accomplishing this goal is to reduce the herbage N concentration in the plant at the time of defoliation by animals (Chapman *et al.* 2014). This would reduce dietary herbage N intake and potentially lower N loading rates in the urine patch. The urine patch is an important contributor to nitrate leaching as large amounts of N from the urine is not able to be taken up by herbage and is therefore, lost through the soil profile into the waterways. High N diets for animals can cause an excess of N relative to animal requirements which is either converted to urea and excreted mainly in the urine, or in dung (Castillo *et al.* 2001; Totty *et al.* 2013). The relationship between N intake and N excretion in urine was shown by Moorby (2014), where an increase in urinary N output in lactating cows increased as N intake increased, with the increase pronounced once N intake exceeded 397 g N/animal/day. In addition to herbage N concentration, the chemical composition of plants may have an impact on the amount of N lost in urine. For example, herbage containing high water soluble carbohydrate (WSC) concentration relative to crude protein (WSC:CP ratio) has been suggested to allow the rumen to capture more N (Edwards *et al.* 2007). While herbage N concentration and chemical composition have been well described for perennial ryegrass (*Lolium perenne L.*) white clover (*Trifolium repens L.*) pastures, less information is available for alternative pasture forages such as the herbs plantain (*Plantago laceloata L.)* and chicory (*Cichorium intybus L.*), legumes lucerne (*Medicago*

sativa L.) and red clover (*Trifolium pratense L.*), and alternative grasses prairie grass (*Bromus willdenowii Kunth)*, Italian ryegrass (*Lolium multiflorum L.)*, tall fescue (*Festuca arundinacea Schreb.),* tetraploid, diploid and high sugar perennial ryegrasses (*Lolium perenne L.*) and cocksfoot (*Dactylis glomerate L.*).

Whilst N concentration of forages affect the dietary N intake, the solubility of the herbage N determines how the dietary N is used in the animal and partitioned into either urine, faeces or animal products (e.g. milk and meat), (Holmes *et al.* 2007b; Pichard and Van Soest 1977). A diet containing high amounts of soluble N inefficiently captures N in the rumen, resulting in higher amounts of urinary N excreted by the animal (Castillo 2001; Pacheco and Waghorn 2008). Perennial ryegrass is high in soluble N (Bryant *et al.* 2012; Hoekstra *et al.* 2008; Nowakowski and Byers 1972). Consequently, forages containing less soluble N are more favoured to reduce N losses because the N is slower to degrade allowing rumen microbes to capture more N for animal production and growth rather than being lost in the urine (Stout *et al.* 1997). Whilst experiments have been carried out using perennial ryegrass (Bryant *et al.* 2012; Hoekstra *et al.* 2007) and white clover (Brown and Pitman 1991; Williams 1955), few studies on herbage N solubility have been carried out on alternative pasture forages such as herbs, legumes and alternative grasses.

Regrowth interval may also be a management tool to change the herbage N concentration and chemical composition of pastures (Blaser 1964; Bryant *et al.* 2012; Hill *et al.* 2005; Mills *et al.* 2009; Peyraud and Astigarraga 1998). For example, in perennial ryegrass it has been shown that, as the regrowth interval increases, herbage N concentration decreases (Bryant *et al.* 2012; Mills *et al.* 2009). Thus, timing of grazing could be used as a management strategy to manipulate herbage N intake and potential urinary N excretion. However, in order to maintain production and profitability of a farm system, the modification of N composition in the herbage must be achieved without a reduction in herbage DM yield and quality. In perennial ryegrass, a longer regrowth interval results in higher herbage DM yields, however the herbage maybe lower in quality (Buxton 1996; Rawnsley *et al.* 2002), hence resulting in lower livestock performance and a loss in profit. However, few studies have examined the effects of regrowth interval on alternative pasture forages such as herbs, legumes and alternative grasses.

Reducing the amount of N fertiliser applied to the farm system is another approach to reducing nitrate leaching whilst maintaining profitability (Chapman *et al.* 2014). The basis of this approach is that forages can be identified that demand lower N fertiliser to achieve similar herbage DM production, making them more efficient at utilising N for growth. Perennial ryegrass pastures usually range in DM response rates to N fertiliser between 5-15 kg DM per kg N applied (kg DM/kg N) (Dairy NZ 2017; Kemp *et al.* 1999a; O'Connor 1982), with a decreasing response as N fertiliser rate increases. However, less

is known about N response rates in alternative pasture forages in a mixture or monoculture. Additionally, previous research has shown plants eventually reach a point of maximum yield where they stop growing regardless of high N fertiliser rates (Ball and Field 1982; Cameron *et al.* June 2005; Cassman *et al.* 2002). At this point, the plant takes up 'luxury' amounts of N from the soil which are not needed, equating to excess N in the plant and inefficient use of N fertiliser.

1.2 Objectives

The main objective of this research was to quantify, for a range of pasture forages (plantain, chicory, lucerne, red clover, white clover, prairie grass, Italian ryegrass, tall fescue, tetraploid perennial ryegrass, diploid perennial ryegrass, high sugar perennial ryegrass and cocksfoot), herbage DM yield, chemical composition, N solubility and herbage N fertiliser requirements in response to different rates of N fertiliser, and regrowth intervals under irrigated pastures in Canterbury.

Specific objectives were to:

1. Quantify the effect of N fertiliser rate on herbage DM yield and chemical composition, in plantain, chicory, lucerne, red clover, white clover, prairie grass, Italian ryegrass, tall fescue, tetraploid perennial ryegrass, diploid perennial ryegrass, high sugar perennial ryegrass and cocksfoot at the optimum defoliation time over a two-year period.

2. Quantify the effect of N fertiliser rate on herbage DM yield and chemical composition in plantain, chicory, red clover, white clover, diploid perennial ryegrass and cocksfoot at different regrowth intervals in spring and autumn.

3. Determine the herbage N concentration and N fertiliser requirements in diploid perennial ryegrass, high sugar perennial ryegrass, Italian ryegrass, cocksfoot, prairie grass, chicory and plantain at maximum herbage DM yield under glasshouse conditions over a six-month summer and autumn period.

4. Quantify the effect on N fertiliser rate on chemical composition and N solubility in plantain, chicory, lucerne, white clover, diploid perennial ryegrass and cocksfoot at optimum defoliation time in the summer and autumn.

These objectives will be examined in four experiments.

1.3 Hypothesis

The following hypothesis were tested in four experiments comparing alterative forages (plantain, chicory, lucerne, red clover, white clover, prairie grass, Italian ryegrass, tall fescue, tetraploid perennial ryegrass, diploid perennial ryegrass, high sugar perennial ryegrass and cocksfoot) to perennial ryegrass and white clover. These were examined as hypothesis that could potentially reduce N loss either through herbage N concentration or plant N use efficiency. The chapters in which each hypothesis is tested can be found in Table 1.1.

Hypothesis #1: *In comparison to perennial ryegrass and white clover, alternate forages have greater DM yield and DM response to N.*

Hypothesis #2: *In comparison to perennial ryegrass and white clover, at optimal time of harvest, alternative pasture forages are lower in herbage N concentration.*

Hypothesis #3: *In comparison to perennial ryegrass and white clover, alternative pasture forages have decreased N solubility and higher WSC:CP ratio.*

Hypothesis #4: *In comparison to perennial ryegrass and white clover, the optimum time to harvest alternative pasture forages to reduce potential leaching losses and obtain high farm production is after 4 weeks of regrowth.*

Hypothesis #5: *In comparison to perennial ryegrass and white clover, lower N fertiliser rates decrease herbage N concentration of alternative pasture forages.*

Hypothesis #6: *In comparison to perennial ryegrass and white clover, lower N fertiliser rates decrease N solubility and increase WSC:CP ratio of alternative pasture forages.*

Table 1.1 Diagram representing thesis structure with objectives and hypothesis of the research presented in this thesis.

Chapter 1	General introduction	
Chapter 2	Literature review	
Chapter 3	**Objective:** Quantify the effect of N fertiliser rate on herbage DM yield and chemical composition, in plantain, chicory, lucerne, red clover, white clover, prairie grass, Italian ryegrass, tall fescue, tetraploid perennial ryegrass, diploid perennial ryegrass, high sugar perennial ryegrass and cocksfoot at optimum defoliation time over a two-year period.	Hypothesis # 1, 2, 5, 6
Chapter 4	**Objective:** Quantify the effect of N fertiliser rate on herbage DM yield and chemical composition in plantain and chicory, red clover, white clover, diploid perennial ryegrass and cocksfoot at different regrowth intervals in spring and autumn.	Hypothesis # 1 - 7
Chapter 5	**Objective:** Determine the herbage N concentration and N fertiliser requirements in diploid perennial ryegrass, high sugar perennial ryegrass, Italian ryegrass, cocksfoot, prairie grass, chicory and plantain at maximum herbage DM yield under glasshouse conditions over a six-month summer and autumn period.	Hypothesis # 1, 2, 5, 6
Chapter 6	**Objective:** Quantify the effect on N fertiliser rate on chemical composition and N solubility in plantain, chicory, lucerne, white clover, diploid perennial ryegrass and cocksfoot at optimum defoliation time in the summer and autumn.	Hypothesis # 2, 3, 6, 7
Chapter 7	General discussion	Hypothesis # 1 - 7

1.4 Thesis structure

This thesis is presented in seven chapters (Table 1.1). In Chapter 2, a literature review concerning the role of N fertiliser and regrowth interval in agricultural systems and their effects on herbage DM yield, chemical composition and N solubility of a range of species is reviewed. Chapter 3 reports on an outdoor plot experiment, conducted over two years, investigating the effects of N fertiliser on herbage DM yield and chemical competition of 12 alternative pasture forages (plantain, chicory, lucerne, red clover, white clover, prairie grass, Italian ryegrass, tall fescue, tetraploid perennial ryegrass, diploid perennial ryegrass and high sugar perennial ryegrass and cocksfoot) under cutting management. Chapter 4 reports on an outdoor plot experiment measuring the effect of N fertiliser and regrowth interval on herbage DM yield and chemical composition of six alternative pasture forages (plantain and chicory, red clover, white clover, diploid perennial ryegrass and cocksfoot) over two seasons (autumn and spring). Chapter 5 reports on an indoor pot experiment carried out to measure N concentration and N fertiliser requirements in seven alternative pasture forages (diploid perennial ryegrass, high sugar perennial ryegrass, Italian ryegrass, cocksfoot, prairie grass, chicory and plantain) at maximum herbage DM yield. Chapter 6 reports on a laboratory experiment using forage samples from the outdoor plot trial to measure the effect of N fertiliser on N solubility and the WSC:CP ratio in six alternative pasture forages (plantain and chicory, lucerne, white clover, perennial ryegrass and cocksfoot) over two seasons (summer and autumn). Finally, Chapter 7 contains the general discussion which combines all four experiments together and includes implications and limitations to the experiments as well as suggestions for future work. The chapters, and their objectives and hypothesis can be found in Table 1.1.

Chapter 2

Literature review

2.1 Introduction

New Zealand's temperate maritime climate favours a pastoral based agricultural system. In this pasture-based system, it is important to maximise herbage dry matter (DM) yield and quality for optimum livestock production. Pastures are typically based on perennial ryegrass (*Lolium perenne*) – white clover (*Trifolium repens)*, with nitrogen (N) fertiliser used to meet plant requirements and increase supply in seasonal deficits such as late autumn and early spring. This often results in increased herbage DM yield and promotes higher stocking rates as the increased feed supply supports increased demand. However, this intensification has brought with it environmental challenges, particularly N losses from farms either as a greenhouse gas, nitrous oxide (NO_2), or as a water pollutant, nitrate (NO_3^-) (Monaghan *et al.* 2007; Valentine and Kemp 1999). For these reasons, regulations developed by regional councils throughout New Zealand have placed limits on the amount of nitrate leached from agricultural land (Ministry for the Environment 2014). These regulations may result in significant reductions in farm productivity and mitigation options which can be rapidly adopted are required (Bryant *et al.* 2007).

A primary reason for the high N losses in pastoral systems is that, due to N fertiliser use and the presence of legumes such as white clover in pastures, contain a high N concentration ranging between 2.9 % N (during early lactation of dairy cows in spring) and 3.7 % N (during late lactation of dairy cows in autumn) (Box *et al.* 2016; Box *et al.* 2017; Clement *et al.* 2016; Moir *et al.* 2007). Although white clover supplies pasture with N through N_2 fixation, a substantial amount of N taken up by plants is from N fertilisers which are applied to maintain productivity of these intensive systems. This contributes to a high intake of N in the herbage, that is excess to animal requirements (Cameron *et al.* 2013; Pacheco and Waghorn 2008; Tamminga 1992). Consequently, large amounts of N are deposited in urine and the subsequent drainage of N from the urine through the soil profile leads to high nitrate leaching. In addition, New Zealand farming systems have long applied large amounts of N fertiliser to boost herbage DM yields, particularly in periods of feed deficit. However, large DM responses to N fertiliser from perennial ryegrass - white clover pastures has led to the over use and dependence on N fertiliser to manage feed supply (Valentine and Kemp 1999). This has led to decreasing N response rates, increased herbage N concentrations and a build-up of mineralisable N in the soil, leading to high N losses (Malcolm *et al.* 2014). To combat this issue, identifying forages that produce similar herbage DM yields with lower N fertiliser requirements and lower herbage N concentrations could be used to mitigate nitrate leaching in farm systems.

Considering the effects animals and plants have on nitrate leaching in agricultural systems, the focus for this review of literature was to investigate the effects of N fertiliser and regrowth interval on herbage DM yield and chemical composition of plants, so they can be used as possible management strategies to mitigate excess N in farm systems.

2.2 Nitrogen cycling in New Zealand farm systems

The transfer of N from one form to another in agricultural systems is generally referred to as the N cycle (Figure 2.1). Whilst N inputs (fertiliser, legume N fixation and animal manure) can be transferred into beneficial products in pasture based systems (crops, milk and meat), they can also act as pollutants (nitrate (NO_3^{-1}) leaching, nitrous oxide (N_2O) emissions), that reduce water and air quality and lead to health hazards, eutrophication and increased greenhouse gas emissions (McLaren and Cameron 1996b).

Figure 2.1 The N cycle in agricultural systems from McLaren and Cameron (1996b).

2.2.1 The nitrification process and nitrate leaching

Nitrogen is easily lost from soil because of its chemical form of nitrate which departs the soil into drainage water and enters rivers, streams and aquifers via groundwater. This causes elevated nitrate levels and increases pollution (Cameron *et al.* 2013). However, there are several processes in which N is converted from urinary or fertiliser N in the soil, to nitrate leached into waterways. Initially, N as the organic form of urea, is decomposed into ammonia (NH_3) in the soil by soil bacteria using the urease enzyme (McLaren and Cameron 1996b):

$(NH_2)_2CO + H_2O \longrightarrow CO_2 + 2NH_3$

It is then hydrolysed into ammonium (NH_4), becoming plant available. This process is called ammonification:

$NH_3 + H_2O \longrightarrow NH_4 + OH^-$

The ammonium in the soil is transformed into nitrate by the process nitrification. This is an aerobic soil process where ammonium is converted into nitrite (NO_2^-) and then further to nitrate (NO_3^-). In the first step of the reaction ammonium is converted to nitrite by bacteria such as *Nitrosomonas* and *Nitrospira* (Cameron 1992):

$2NH_4^+ + 3O_2 \longrightarrow 2NO_2^- + 2H_2O + 4H^+ + \text{energy}$

In the second step nitrite is oxidized by *Nitrobacter* bacteria to create nitrate (Cameron *et al.* 2013; Di *et al.* 2010):

$2NO_2^- + O_2 \longrightarrow 2NO_3^- + \text{energy}$

Nitrate is negatively charged and therefore repelled from soil particles because they are also negatively charged (Cameron 1992; Fertresearch 2011). As a result, nitrate is plant available but also easily leached through the soil profile during a drainage event such as high rainfall or irrigation (McLaren and Cameron 1996c). This is unlike ammonium, which has a positive charge and therefore binds to soil particles allowing it to be readily available in the soil for plant uptake.

2.2.2 **Nitrogen in animals**

For animals, dietary N is required for the synthesis of body tissues, wool, milk enzymes and hormones (Brookes and Nicol 2007). However, when a high N diet is fed, this provides an excess of N relative to animal requirements (Cameron *et al.* 2013; Pacheco and Waghorn 2008; Tamminga 1992). In the rumen, N is broken down by microbes into a form that can be absorbed for growth and production (Holmes *et al.* 2007b). However, high N feeds provide surplus N, which microbes are not able to process quickly enough into microbial protein. As a result, the build-up of N in the rumen becomes toxic and to prevent N toxicity, the dietary N is converted into ammonia and absorbed into the bloodstream. Upon entering the bloodstream, ammonia is transported to the liver where it is converted to urea and either recycled via saliva, passed out in the dung or more typically excreted in the urine (Castillo *et al.* 2001; Totty *et al.* 2013). Consequently, efficiency of N utilisation (NUE) in the animal is higher when fed lower dietary N because the microbes in the rumen are more likely to break down the N into a form which can be absorbed for growth and production rather than being lost in the urine.

The relationship between a lower N diet and the decrease in urinary N loss was shown by Moorby (2014) where, urinary N output was higher when lactating cows were fed a higher proportion of perennial ryegrass - white clover pasture in their diet. Up to a herbage N intake of 397 g N/animal/day, the mean N output was low (81 g N/day) meaning dietary N was within the animal's ability to utilise it. However, in this instance, above a herbage N intake of 397 g N/animal/day the urine N output increased significantly, demonstrating N intake was surplus to animal requirements and therefore converted into urinary or faecal N and excreted (Figure 2.2). The area where the urinary N is deposited on the paddock is known as a urine patch and normally contains N loads that exceed the pastures ability to fully utilise N before leaching occurs. Therefore, urine patches are the main cause of nitrate leaching from typical pasture grazing systems (Di and Cameron 2000; Haynes and Williams 1993; Ledgard *et al.* 2009; Ledgard 2001; Ryden *et al.* 1984). The risk of surplus N is higher following peak lactation in spring when animal demand for dietary N declines with milk yield. Consequently, if the N concentration of summer or autumn herbage exceed animal requirements, the risk of N loss in the urine is higher, increasing the amount of excess N in the farm system.

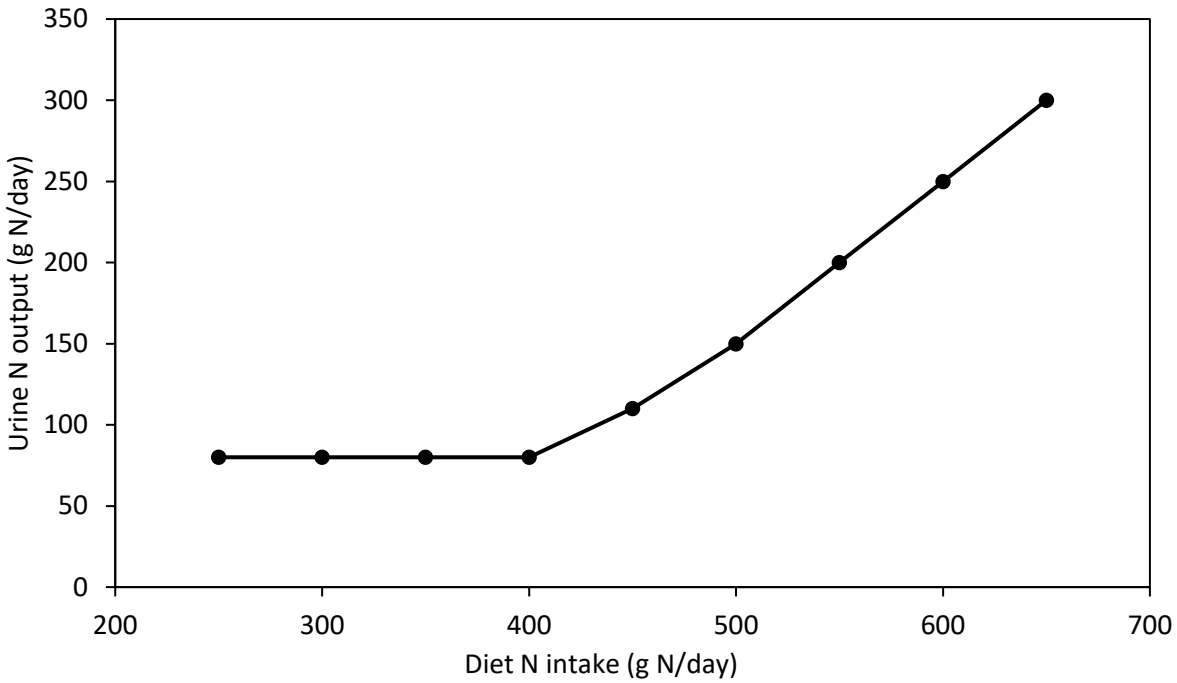

Figure 2.2 Relationship between herbage N intake and urine N output in individual cows fed diets based on fresh grass (adapted from Moorby (2014)).

2.2.3 Nitrogen in plants

For plants, N is essential for many functions and a primary constituent of amino acids (AAs) which are the building blocks of proteins (McLaren and Cameron 1996b). It is needed for photosynthesis and is also an essential element for various enzymes for high herbage DM yields and development. For these

reasons, N fertiliser allows farmers to achieve high yielding pastures essential for superior production and profit. Thus, N is the most commonly used fertiliser nutrient to increase herbage DM yields of perennial ryegrass-white clover pastures (Ball *et al.* 2012). Initially, N taken up by the plant from the soil which increases herbage N concentration in the plant through luxury uptake in the roots. This causes an increase in cell elongation and leaf area of the plant, a higher number of tillers and increases DM yield. As a result, after 2 – 3 weeks, the herbage N slowly decreases due the dilution N in the plant returning N concentration back to the original value (Ball *et al.* 2012). Nitrogen can be provided to the soil and plants via applying N fertiliser or by biological N fixation from legumes within the pasture (McKenzie *et al.* 1999; Mills and Moot 2010). However, the over application of N fertiliser may result in an inefficiency of N in the plant and an increased risk in nitrate leaching (Cassman *et al.* 2002). This is because of plant growth responses, which can be described as a Mitscherlich type DM response curve (Figure 2.3 (a)), where plants eventually do not grow any further because they reach a maximum herbage DM yield potential. After the point of maximum herbage DM yield, the N in the plant becomes saturated but the plant may still take up 'luxury' amounts of N, that is not needed (Smith *et al.* 1985), (Figure 2.3 (b)). As a result, excess N in the plant occurs, causing higher herbage N intake by animals and higher urinary N losses. Alternatively, when lower growth rates occur in pastures, e.g. in the autumn and winter, N is not taken up by plants and instead remains in the soil as mineralisable N which is easily leached in a high rainfall event (Malcolm *et al.* 2014). This can also be described as low plant N use efficiency (NUE) which is measured as the herbage DM response to N fertiliser applied (kg DM grown/ kg N applied). One way to improve plant NUE in farm systems, is to identify forages that demand less N fertiliser and herbage N requirements required to obtain similar herbage DM yield of a plant. The value of N at which this occurs is otherwise known as critical N concentration and can be described in more detail as the amount of N required for plants to reach a threshold slightly less than maximum growth e.g. 90% of the maximum yield or optimum growth (Chapman *et al.* 2014). This is broken into critical internal N concentration (N_{CInt}); also known as herbage N content, where N is a measure of plant tissue N (%) at slightly less than maximum yield (Figure 2.3 (a)) and critical external N concentration (N_{CExt}); where N is a measure of soil N content or N fertiliser supply (kg N applied) at slightly less then maximum yield (Figure 2.3 (b)).

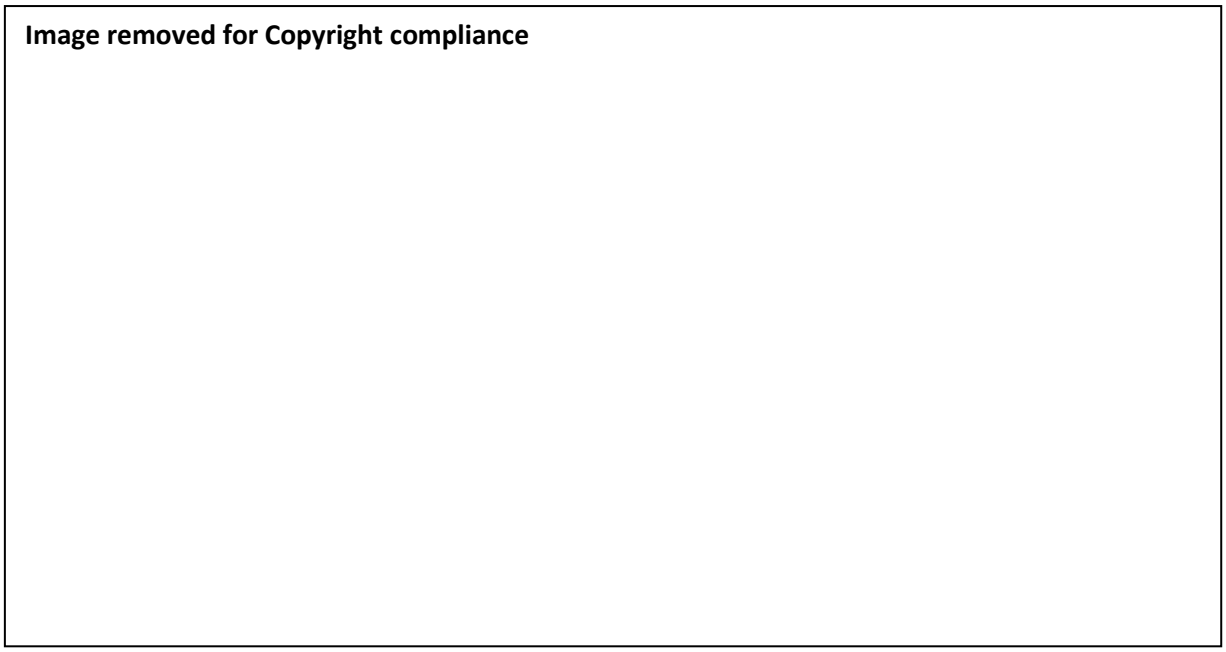

Figure 2.3: Effect of (a) increasing concentrations of nitrogen in nutrient solution, and (b) increasing concentrations of nitrogen in the shoots, on relative herbage DM yield of perennial ryegrass (*Lolium perenne*) grown in sand. From Smith et al. (1985).

Attempts to breed perennial ryegrass cultivars with lower herbage N concentration have met with limited success (Wilkins *et al.* 2000); however Hill *et al.* (2005) found differences in plant N content of up to 13.5 g/kg among nine different grass species (Table 2.1). Variation among species is most likely due to the differences in metabolism, storage and mobilisation of herbage N among the species and demonstrates the differences in N cycling among the species and the plants demand for fertiliser N, which is higher in some pastures than others at 90% DM yield (Table 2.1). However, limited work has been carried out investigating the critical herbage N concentrations and N fertiliser demand at maximum yield in a wide variety of forages.

Table 2.1 Critical herbage N and N fertiliser/soil N requirements for nine grass forages from southern Australia pastures (Hill et al. 2005).

Forage	Scientific name	Critical herbage N concentration (% of DM)	Critical N fertiliser or soil N (milligram of N per pot (mg N/pot))
Yorkshire fog	*Holcus lanatus*	2.08	76
Capeweed	*Arctotheca calendula*	1.35	88
Wallaby grass	*Olearia viscidula*	1.8	93
Weeping grass	*Microlaena stipoides*	1.53	102
Silver grass	*Miscanthus sinensis*	2.15	109
Phalaris	*Phalaris aquatica*	1.72	114
Smooth brome grass	*Bromus inermis*	1.96	125
Wimmera ryegrass	*Lolium rigidum*	2.67	138
Barley grass	*Hordeum leporinum*	1.69	164

2.3 Mitigation strategies to reduce nitrate leaching in farm systems

Mitigation strategies to reduce nitrate leaching from grazed pasture systems while maintaining profitability are highly sought after and have been discussed in a number of reviews (Bryant *et al.* 2007; Di and Cameron 2002a; Kebreab *et al.* 2001; Ledgard *et al.* 2006b). In these reviews, many different approaches to reduce nitrate leaching have been shown including management of soil, stocking rate, stand-off pads, forage type, regrowth interval and fertiliser rate. Whilst a brief discussion on soils and animal distribution is provided, the focus for this literature review is to discuss mitigation strategies to reduce herbage N intake by livestock and soil mineralisable N left in the soil. This is through the manipulation of forage DM response to N and chemical composition through the management of N fertiliser and regrowth interval.

Light sandy based soils with poor soil texture and structure such as pumice (McLaren and Cameron 1996a), are likely to leach more nitrate than heavier clay based soils such as gley (McLaren and Cameron 1996a), under the same climate conditions (Bergström and Johansson 1991; Garwood and Ryden 1986; McLaren and Cameron 1996b). This is because heavy soils have a small soil particle size and tight structure with poor drainage, meaning nitrate is less likely to leach out of the profile. Light soils however, have large particles and a loose structure creating large pores which water and nitrate can easily drain through and out of the soil profile causing nitrate leaching. An example of this can be found in Bergström and Johansson (1991) where the largest leaching losses of nitrate (65 kg/ha/year) occurred in the sandy soil and the smallest leaching losses (20 kg N/ha/year or less) occurred in a clay soil.

As the number of urine patches and N loading are major contributors to nitrate leaching (Di and Cameron 2000; Haynes and Williams 1993; Ledgard et al. 2009; Ledgard 2001; Ryden et al. 1984), one mitigation strategy to reduce N loss in light soils is to reduce the stocking density. In Moir et al. (2011) when stocking density was less intensive (measured as annual cow grazing hours), the number of urine patches were lower (Figure 2.4), thus reducing the amount of N loading and nitrate leaching onto the area. However, this may result in lower profitability on farm due to fewer cows producing less milk and issues with maintaining pasture quality (Valentine and Kemp 1999). This was examined by Clement et al. (2016) where in the low N loading treatment was 33 % lower in urinary N excretion per ha in comparison to the High N loading treatment. However, milk solid (MS) production was 24 % lower in the low N loading treatment (2242 kg MS/ha vs 1700 kg MS/ha). A series of recent studies by Chapman et al. (2017a) have examined this concept. It was shown that a lower stocking rate system leached lower amounts of N and had an increase in MS production per cow and total operating profit ($ 4,302/ha vs. $4,205/ha) due to lower N fertiliser rates, less supplementary feed bought and lower animal health and cow related costs (Table 2.2).

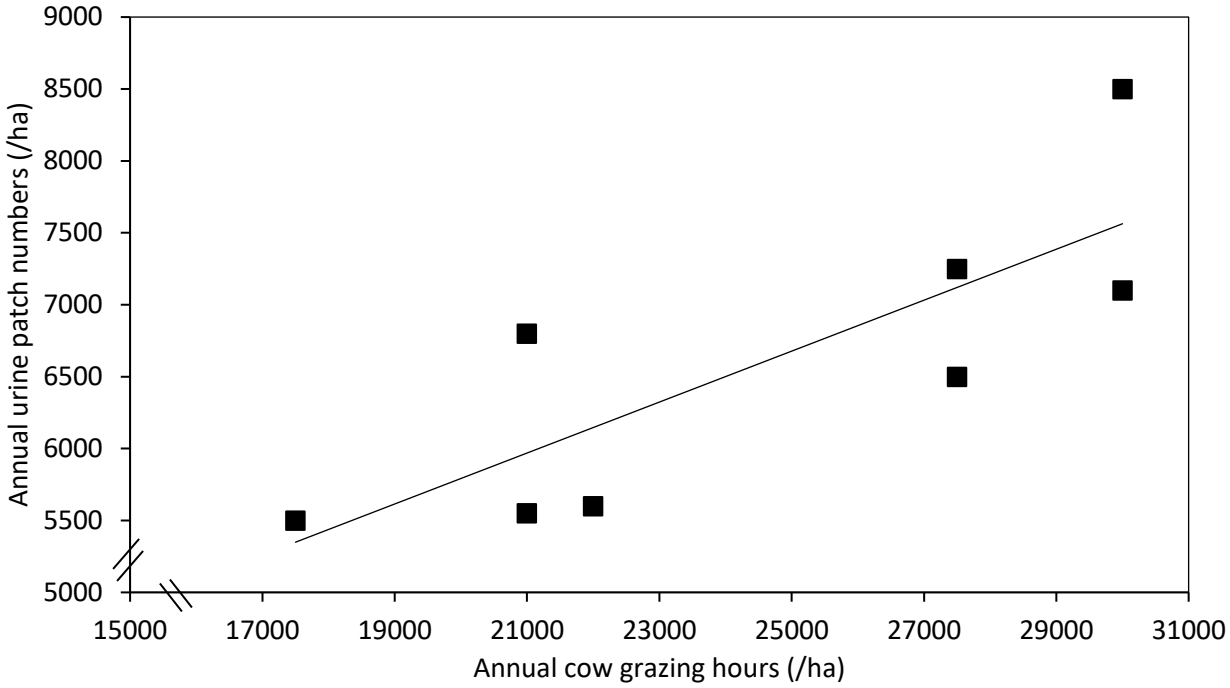

Figure 2.4 The relationship between total annual cow grazing hours/ha and annual urine patch numbers/ha for all years. n = 8, P = 0.01 (adapted from Moir et al. (2011)).

Table 2.2 Observed physical production, profit and nitrate leaching for the "lower input, high efficiency" (LIHE) and "higher input, high efficiency" (HIHE) milking platform (MP) systems in Canterbury (Chapman *et al.* 2017a).

	Low input, high efficiency	Higher input, high efficiency
Stocking rate (cows/ha)	3.5	5.0
N fertiliser applied (kg N/ha)	154	309
Pasture for grazing (t DM/cow)	4.83	3.66
Supplementary feed (t DM/cow) (Incl. grain, silage, winter crop)	1.41	2.34
MS production per cow (kg)	509	476
Operating profit ($/ha)	4302	4205
N leached (kg/ha)	32 (27-35)	46 (40-52)

A further alternative is to use infrastructure to remove animals from pasture to decrease N leached using stand-off pads (Ledgard *et al.* 2006a; Ledgard *et al.* 2006b; Monaghan *et al.* 2007). These may be especially useful in the winter when high stocking density used to graze high herbage DM yield of winter crops, combined with high rainfall and subsequent drainage, leads to large nitrate leaching losses (Chrystal *et al.* 2016). This nitrate leaching, during an eight-week winter period, accounts for 11-24 % of annual farm N losses (Chrystal *et al.* 2016). The stand-off pad concept works by capturing into the effluent system, the highly concentrated urinary N normally deposited onto pasture, and redistributing it back onto paddocks at a lower N rate, and at times of year that are lower risk to nitrate leaching (Christensen *et al.* 2012b). As a result of timing and rate of application, N is utilised more efficiently by the pasture. This mitigation strategy was shown to be the most effective at reducing nitrate leaching in a study carried out by Christensen *et al.* (2012a) where, a 36 % reduction in total N

losses was reported when lactating dairy cows were removed from pasture for 20 hours per day compared with a standard grazing system. In addition, an analysis carried out by De Klein and Ledgard (2001) using OVERSEER® where, paddocks under a nil grazing regime during the autumn/winter months (April to August) showed nitrate leaching losses may be reduced by 35-50 %. Other benefits of using stand-off pads include reduced pugging damage, improve animal welfare and increased efficiency in the use of supplements (Monaghan *et al.* 2007). However, the capital cost of a stand-off pad is significantly higher than wintering on pasture and may also cause pasture quality issues in late autumn/winter when animals cannot graze the pasture.

Other strategies proposed to reduce N losses in the farm system include altering herbage chemical composition, herbage N solubility and plant N use efficiency (NUE) of pastures grazed by animals through manipulating different management practices such as regrowth interval, forage type and N fertiliser application.

Some work has been done on diverse pastures containing chicory and plantain which suggests, due to their lower herbage N concentrations, N leaching is therefore lower when grazing these forages. For example, a study carried out by Totty et al. (2013) showed urinary N excretion was 19.3 % lower in cows grazing a diverse pasture mixture (3.8 % N), than cows fed ryegrass-white clover (4.2 % N). In addition, Beukes *et al.* (2014) predicted N leaching in urine patches, was 19 % less when animals grazed 50% diverse pastures (3.3 % N), compared to when animals grazed 100 % ryegrass-white clover which contained (3.4 % N). This was calculated using the Molly cow model and data from Nobilly *et al.* (2013) (Canterbury) and Woodward *et al.* (2013) (Waikato).

2.3.1 **Herbage DM yield and N concentrations**

Maintaining high herbage DM yield is critical to achieving high livestock production in agricultural systems (Holmes *et al.* 2007a; Thomson and Poppi 1990). The excessive application of N fertiliser to achieve this, has resulted in the inefficiency of N use in perennial ryegrass –white clover pastures (Ball and Field 1982; Cameron *et al.* June 2005; Cassman *et al.* 2002; Smith *et al.* 1985). This may cause an increase in nitrate leaching through either increased herbage N intake in the diet or higher mineralisable soil N (Malcolm *et al.* 2014). Therefore, this suggests identifying forages that require lower amounts of N fertiliser to obtain similar herbage DM yields are more sought after to reduce N losses. This is quantified using forage DM responses to N fertiliser and can otherwise be described as plant nitrogen use efficiency (NUE).

Throughout the growing season, perennial ryegrass - white clover pastures contain high N concentrations, which range between 2.9 % N (during early lactation of dairy cows in spring) and 3.7 % N (during late lactation of dairy cows in autumn) (Box *et al.* 2016; Clement *et al.* 2016; Moir *et al.*

2007). These values exceed animal N requirements of 2.8 % N for a dairy cow producing average milk production (2.0 kg MS/day) (AFRC 1993). Surplus dietary N is converted into urea and passed into the urine (Pacheco and Waghorn 2008). Here the N is highly concentrated and therefore causes greater leaching potential from the urine patch. Therefore, identifying forages with different N concentrations at time of grazing or harvest could be a key way to reduce the amount of N in the diet, potentially reducing N losses.

Forage effect

Attempts to breed perennial ryegrass cultivars with high herbage DM yields and lower herbage N concentration have met with limited success. For example, a study by Wilkins et al. (2000) tested the herbage N concentration of eight cultivars of perennial ryegrass, including two high sugar perennial ryegrasses containing high water soluble carbohydrates (WSC), and early, intermediate and late heading varieties. It was found that, although herbage DM yields of some newer varieties were up to 70% more than their respective older varieties with similar heading dates, differences in herbage N concentration between the varieties were too small to have a serious impact of the nutritional value of the herbage. Therefore, as differences between varieties did not show large differences, the use of alternative herb, legume and grass forages that contain lower herbage N concentration and high herbage DM yields could instead be used to reduce nitrate leaching in agricultural systems.

Nitrogen concentrations and herbage DM yields of individual forages have been well documented in grasses, crops and legumes (Burke *et al.* 2000; Fulkerson *et al.* 2008; Fulkerson *et al.* 2007; Labreveux *et al.* 2004; Lee *et al.* 2015; Sanderson *et al.* 2003). Lee *et al.* (2015) showed at defoliation (450 – 550 mm extended leaf height), plantain and chicory contained N concentrations of 2.5 and 3.0 % N, respectively, over an 18-month period and herbage DM yields were 23.0 t DM/ha for chicory and 29.8 t DM/ha for plantain over the 18-month period. Likewise, Sanderson *et al.* (2003) showed chicory and plantain averaged 2.3% N in both forages (Table 2.3) and Labreveux *et al.* (2004) showed plantain was generally higher in herbage DM yield than chicory (9470 kg DM/ha total vs 9023 kg DM/ha total), although not as high as cocksfoot (11,130 kg DM/ha total), (Table 2.4). These N concentration values are lower than perennial ryegrass- white clover pastures, which have N concentrations between 2.9 % N (during early lactation of dairy cows in spring) and 3.7 % N (during late lactation of dairy cows in autumn) (Box *et al.* 2016; Clement *et al.* 2016; Moir *et al.* 2007). In addition, these alternative herb forages still maintain high herbage DM yields. Therefore, they could be used as alternatives to the traditional pastures found on New Zealand dairy farms.

Table 2.3 Nutritive value of chicory (*Cichorium intybus*) and plantain (*Plantago lanceolata*) grown in two field experiments at Rock Springs Pennsylvania, USA. Data for Exp.1 are averages of 3 and 5 week cutting intervals and five replicates. The data for Exp.2 are averages of five replicates. Adapted from Sanderson *et al.* (2003). N concentration calculated using crude protein values x 6.25.

	Exp. 1 (1998)			Exp.2 (2000)	
Cultivar	**May**	**Jun**	**Sep**	**May**	**Sep**
	----------g N/kg DM----------				
Feast chicory	28.0	31.2	25.8	24.2	20.3
Puna chicory	32.0	29.0	24.3	21.6	21.0
Lacerta chicory	-	-	-	19.7	16.6
Lancelot plantain	24.0	27.7	18.9	18.2	16.8
Tonic plantain	29.6	28.6	22.6	21.4	17.3
Contrast – Chicory vs. plantain	NS	**	**	**	*

* significant at P<0.05
** significant at P<0.01
NS not significant

Table 2.4 Seasonal productivity of chicory (*Cichorium intybus*), plantain (*Plantago lanceolata*), and cocksfoot (*Dactylis glomerata*) under grazing during 1998 (Labreveux *et al.* 2004).

Cultivar	Spring 1998	Summer 1998	Total
	----------kg DM/ha----------		
Feast chicory	6,240	2,450	8,690
Lacerta chicory	5,710	2,800	8,510
Puna chicory	6,510	3,350	9,870
Lancelot plantain	7,360	2,690	10,070
Tonic plantain	5,700	3,170	8,870
Pennlate cocksfoot	7,240	3,890	11,130

Herbage N concentrations of alternative grasses, legumes and herbs were also examined in Fulkerson *et al.* (2007) and Fulkerson *et al.* (2008). It was shown some forages contain higher N concentrations than others (Table 2.5). For example, averaged over the year, red clover and prairie grass had the highest herbage N concentrations (44.5 g N/kg DM and 44.2 g N/kg DM, respectively) and paspalum and perennial ryegrass had the lowest herbage N concentrations (34.2 g N/kg DM and 38.7 g N/kg DM).

Table 2.5 N concentration of alternative grasses and legumes during spring, summer, autumn and winter. Adapted from Fulkerson *et al.* (2007)* and Fulkerson *et al.* (2008). N concentration calculated using crude protein values x 6.25.**

Forage	Scientific name	Summer	Autumn	Winter	Spring	Average
		----------g N/kg DM----------				
Perennial ryegrass*	*Lolium perenne*	35.4	38.4	38.9	42.1	38.7
Cocksfoot*	*Dactylis glomerata*	36.5	36.3	51.0	42.7	41.6
Fescue*	*Festuca arundinacea*	34.6	43.0	40.5	42.7	40.2
Phalaris*	*Phalaris aquatica*	38.6	46.6	45.6	43.4	43.5
Prairie grass*	*Bromus wildenowii*	39.4	45.6	49.6	42.2	44.2
Kikuyu*	*Pennisetum clandestinum*	40.5	46.9	-	36.5	41.3
Paspalum*	*Paspalum dilatatum*	-	31.7	-	36.6	34.2
Lucerne *	*Medicago sativa*	33.0	-	48.2	46.4	42.5
Red clover*	*Trifolium pratense*	38.7	-	48.5	46.2	44.5
White clover*	*Trifolium repens*	38.7	-	47.7	45.0	43.8
Chicory**	*Cichorium intybus*	39.8	44.5	46.2	43.7	43.6
Plantain**	*Plantago lanceolata*	-	48.0	39.7	45.0	44.2
Perennial ryegrass**	*Lolium perenne*	38.9	38.4	38.9	42.1	39.6

An *in sacco* trial (Burke *et al.* 2000) was also carried out using 10 forages that were minced to a particle size similar to chewed material. It was found lucerne and white clover contained the highest herbage N concentrations in the plant (4.8 and 3.3 %N, respectively) and paspalum contained the lowest herbage N concentration in the plant (2.2 % N) (Table 2.6).

Table 2.6 Nitrogen concentration of fresh leafy forages prepared by mincing similar to chewing and incubated *in sacco*. Data from Burke *et al.* (2000).

Forage	Scientific name	N Concentration (% of DM)
Chicory	*Cichorium intybus*	3.0
Plantain	*Plantago lanceolata*	4.0
White clover	*Trifolium repens*	4.3
Birdsfood trefoil	*Lotus corniculatus*	3.5
Lucerne	*Medicago sativa*	4.8
Perennial ryegrass	*Lolium perenne*	2.6
Yorkshire fog	*Holcus lanatus*	3.8
Tall Fescue	*Festuca arundinacea*	2.6
Kikuyu	*Pennisetum clandestinum*	2.6
Paspalum	*Paspalum dilatatum*	2.2

Overall, is has been shown that some forages contain lower herbage N concentration and high herbage DM yields than others and therefore, could be used as an alternative to perennial ryegrass white clover pastures to reduce animal N intakes and subsequent nitrate leaching in agricultural systems.

N fertiliser effect

Nitrogen response rates of individual forage DM yields have been well documented in grasses, crops and legumes (King *et al.* 2012; McKenzie *et al.* 1999; Moore *et al.* 1991; O'Connor 1982; Sinclair and Horie 1989; Van Rossum *et al.* 2013). Legumes have a negligible response to N because their N needs

are met though biological N fixation (McKenzie *et al.* 1999; Mills and Moot 2010). However, perennial ryegrass-white clover pastures have an increasing response to N fertiliser, with values on intensively managed pastures usually between 5-10 kg DM/kg N applied, when all other environmental factors are not limiting, averaged across New Zealand (Kemp *et al.* 1999a). Chicory has also been shown to have an increasing DM response to N, with values of 7.3 kg DM/kg N when 200 kg N/ha was applied in the Manawatu (Clark *et al.* 1990). A experiment by Van Rossum *et al.* (2013) using diverse pastures in irrigated Canterbury, pasture containing 20 % chicory and plantain showed a lower response to N fertiliser (0.8 kg DM/kg N) than that of standard perennial ryegrass–white clover pasture (8.0 kg DM/kg N). However, there is little information on N responses in plantain monocultures.

It is difficult to compare forage DM responses to N between experiments because the environment has such a large effect on plant growth. Timing of N application makes a significant difference in DM responses because of temperature (season) and maturity (Ball and Field 1982; Hill *et al.* 2007; O'Connor 1990; White *et al.* 2007). For example, DM responses to N are less in the autumn and winter months (7 kg DM/kg N applied in March – August (Cameron *et al.* June 2005)) than spring and summer (12 kg DM/kg N applied in September – February (Cameron *et al.* June 2005)) because of lower temperatures which restrict plant growth rates and therefore herbage DM yield (Figure 2.5, Figure 2.6). Soil type, cropping history and nutrient availability to the plant also have a significant effect on herbage growth (McLaren and Cameron 1996b). Since the environment has such a large impact on DM responses to N, an experiment comparing annual N responses of a range of forages in a controlled environment is needed to identify whether some have lower N requirements than others.

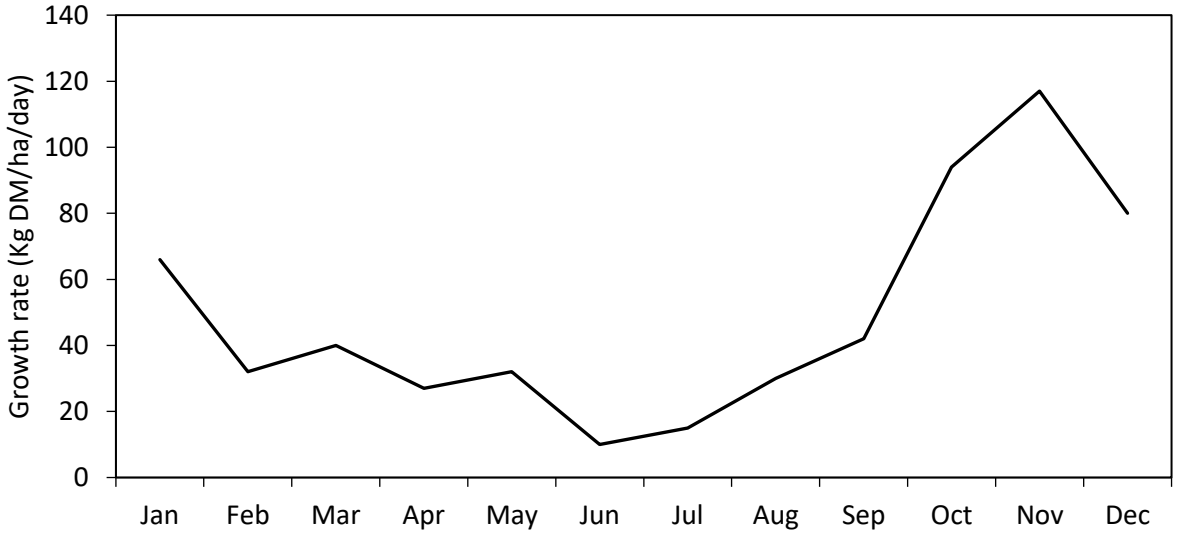

Figure 2.5 Monthly perennial ryegrass – white clover (*Lolium perenne* – *trifolium repens*) pasture growth rates from 2001 – 2003 for Lincoln dairy unit, soil is a light sandy loam, 200 kg N/ha/y applied. Data is based on averages from several years for specific sites. Adapted from Dairy NZ (2017).

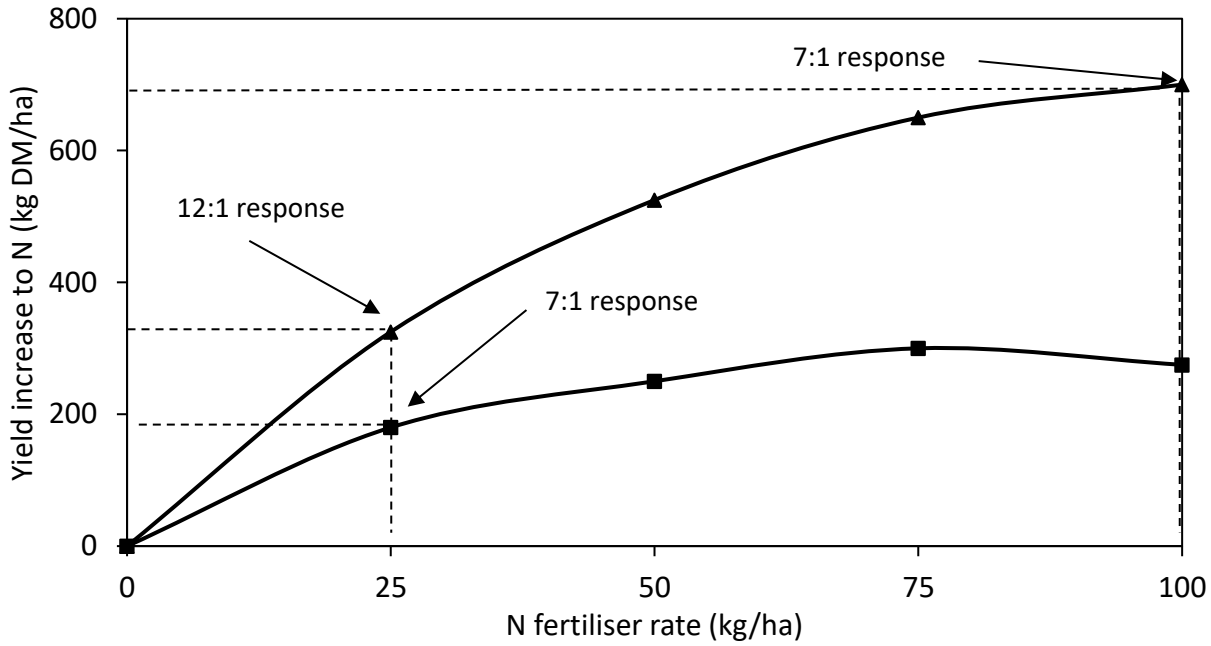

Figure 2.6 Effect of season on N response rate of perennial ryegrass -white clover (*Lolium perenne – trifolium repens*) pasture in the spring (▲) and winter (■). Adapted from Cameron *et al.* (June 2005).

Nitrogen response rates of individual forage N concentrations in grasses have also been well documented (Moir *et al.* 2013; Smith *et al.* 1985; Wilkins *et al.* 2000). Although there is variation between plant N concentrations at different N fertiliser rates, it has been suggested the change is usually small. This was shown in Wilkins et al. (2000), where the variation in plant N concentration between the low (100 kg N/ha/yr) and high (500 kg N/ha/yr) N fertiliser rates was 6 g N/kg DM, averaged over the eight perennial ryegrass species over 2 years (Figure 2.7). However, little is known about N concentrations in alternative pasture forages such as plantain and chicory.

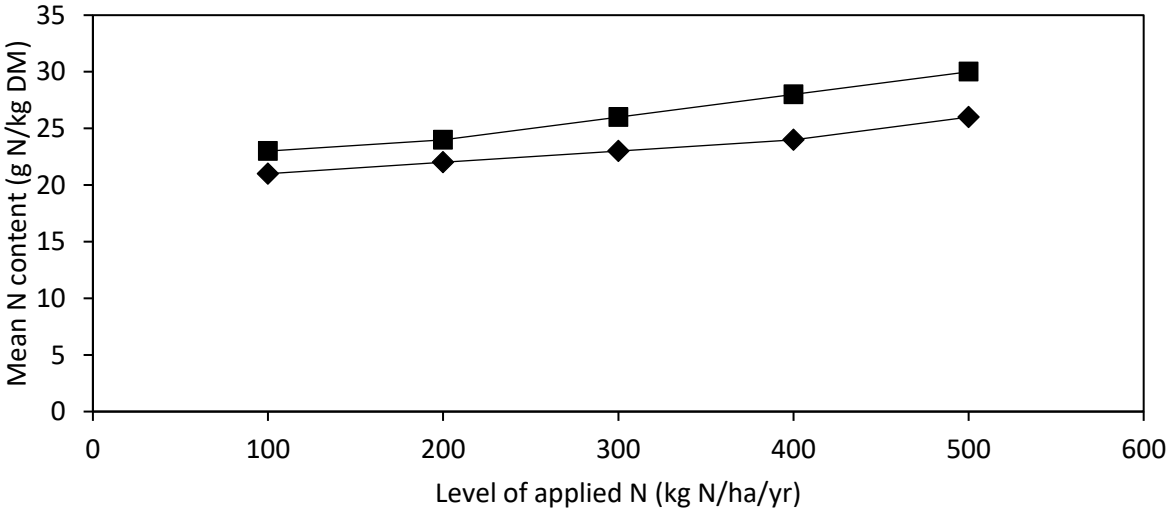

Figure 2.7 Influence of N fertiliser on mean N concentration of eight perennial ryegrass (*Lolium perenne*) cultivars in 1997 (◆) and 1998 (■). Adapted from Wilkins et al. (2000). N concentration calculated using crude protein values x 6.25.

Regrowth interval effect

It is widely documented that in perennial ryegrass pastures, after the application of N fertiliser at start of plant regrowth, an increase in herbage N concentration occurs in the plant through luxury uptake (Ball *et al.* 2012; Bryant *et al.* 2012; Hoekstra *et al.* 2007; Peyraud and Astigarraga 1998; Wilman 1975). At first, after N fertiliser is applied to recently grazed or cut herbage, an increase in herbage N concentration occurs through luxury plant uptake from the soil. This equates to an increase in elongation of cells and the plant leaf area resulting in thinner and longer leaves, and a greater amount of tillering. This is because of the plants ability to intercept light and produce more photosynthates (Woledge 1988). Around 2 – 3 weeks later in the regrowth, due the elongation of the cells, the dilution of N in the plant cells occurs and herbage N concentration decreases (Ball *et al.* 2012; Wilman 1975), (Figure 2.8). Thus, delaying grazing of perennial ryegrass could be used as a mitigation tool to reduce N intake in animals per kg DM (Hoekstra *et al.* 2007; Peyraud and Astigarraga 1998) whilst maintaining high herbage DM yields needed for sufficient farm production.

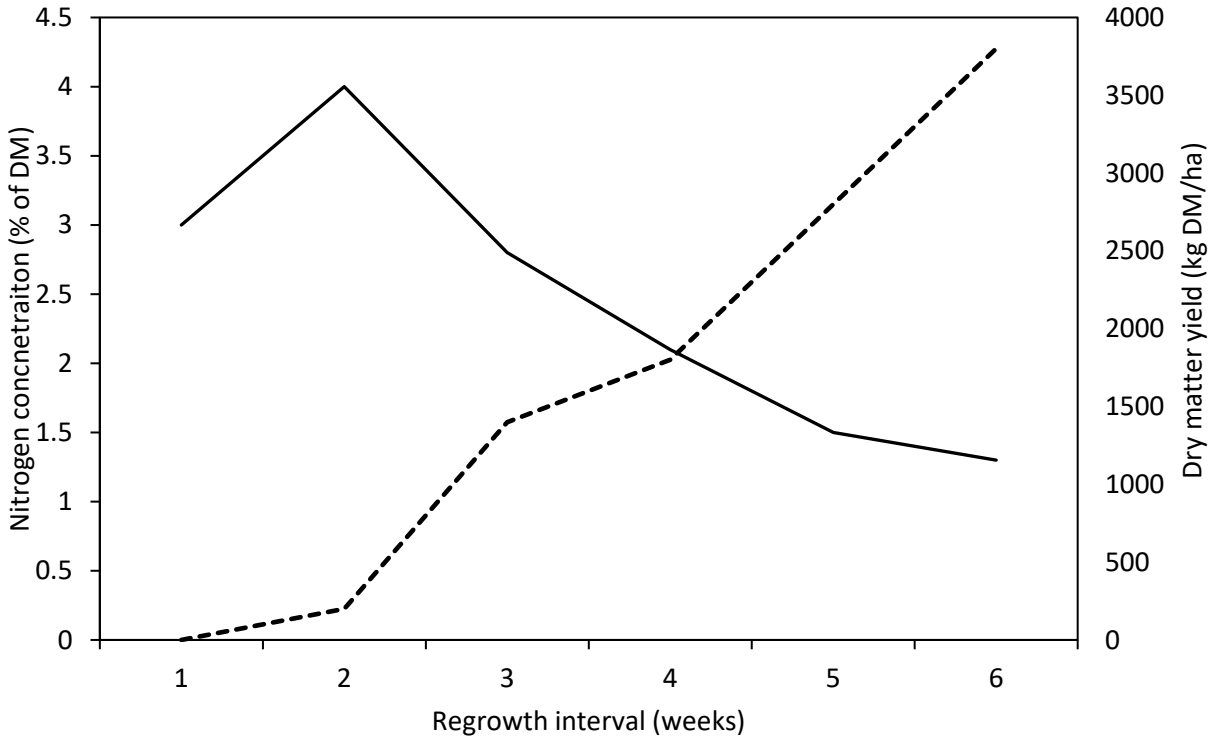

Figure 2.8 N concentration (—) and herbage DM yield (- - -) of Italian ryegrass (*Lolium multiflorum*) after application of 30 kg N/ha. Adapted from Wilman (1975).

A decrease in N concentration as regrowth interval increases, also occurs in herb species as shown in Figure 2.9. As regrowth interval (measured as cutting height) increased from 150 mm to 450/550 mm, herbage DM yield (over the 18 months) increased from 18.8 t DM/ha in chicory and 21.3 t DM/ha in plantain at the lowest defoliation height, to 23.0 t DM/ha in chicory and 29.8 t DM/ha in plantain at the highest defoliation height. Alternatively, N concentrations in the herbs decreased from 4.2 g N/kg

DM in chicory and 3.8 g N/kg DM in plantain at the lowest defoliation height, to 3.0 g N/kg DM in chicory and 2.5 g N/kg DM in plantain at the highest defoliation height.

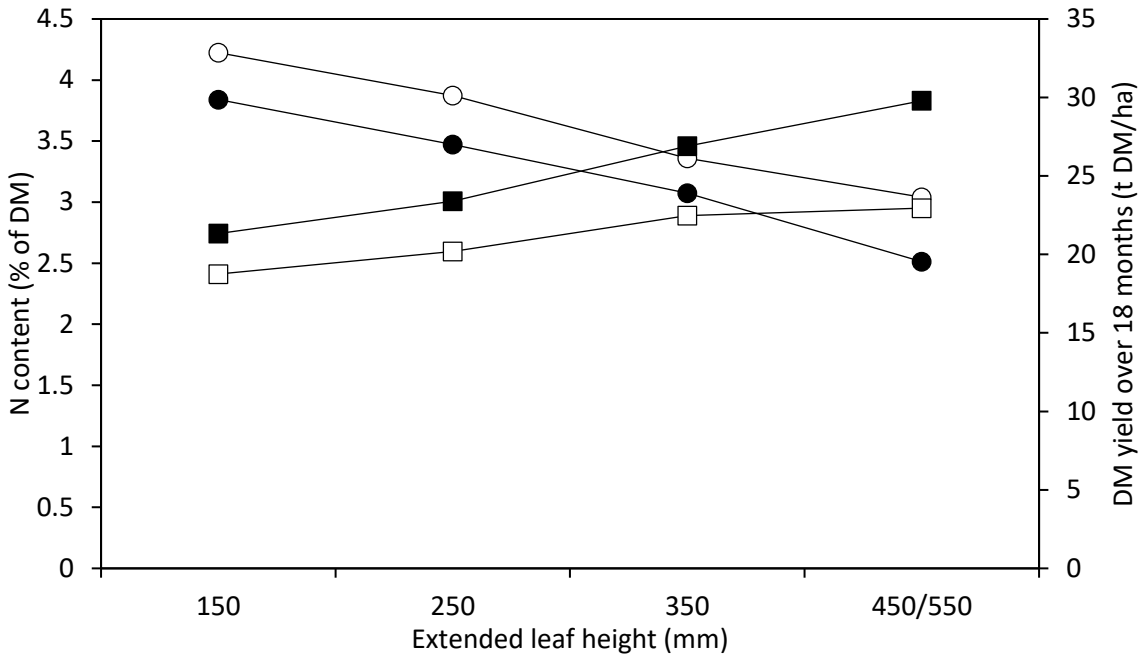

Figure 2.9 N concentration (% of DM) in herbage from spring sown chicory (*Cichorium intybus)* (○) and plantain (*Plantago lanceolata*) (●) swards and herbage DM yield (kg DM/ha/yr) from spring sown chicory (□) and plantain (■) defoliated at extended leaf heights of 150, 250, 205 or 450 or 550 mm over 18 months. Adapted from Lee *et al.* (2015). N concentration calculated using crude protein values x 6.25.

Though extending the regrowth interval may be a good strategy to reduce N losses from agricultural systems, it is important to make sure the forage quality and digestibility, measured by metabolisable energy (ME), does not hinder livestock performance. Plants provide energy needed for essential body functions; a higher quality pasture supplies an animal with more energy thus increasing animal production (Holmes *et al.* 2007b). Thus, a decrease in plant quality, as measured by ME, may decrease livestock performance and therefore farm profitability.

Previous experiments have found the quality of perennial ryegrass usually declines as regrowth interval is extended. This is shown in Figure 2.10 where, as crude protein in plants decreases over time from 15 % in DM to 6 % in DM, the metabolisable energy (ME) also decreases from 12.0 MJ/kg DM to 8.9 MJ/kg DM, decreasing the quality of the pasture (Waghorn and Clark 2004). This is due to increase of neutral and acid detergent fibre (NDF, ADF) from leaf senescence and stem growth (Fulkerson and Donaghy 2001; Hoekstra *et al.* 2007; Waghorn and Clark 2004). Thus, finding a point in the regrowth interval where pasture quality is acceptable for animal production whilst being low in N concentration would be the best time to graze.

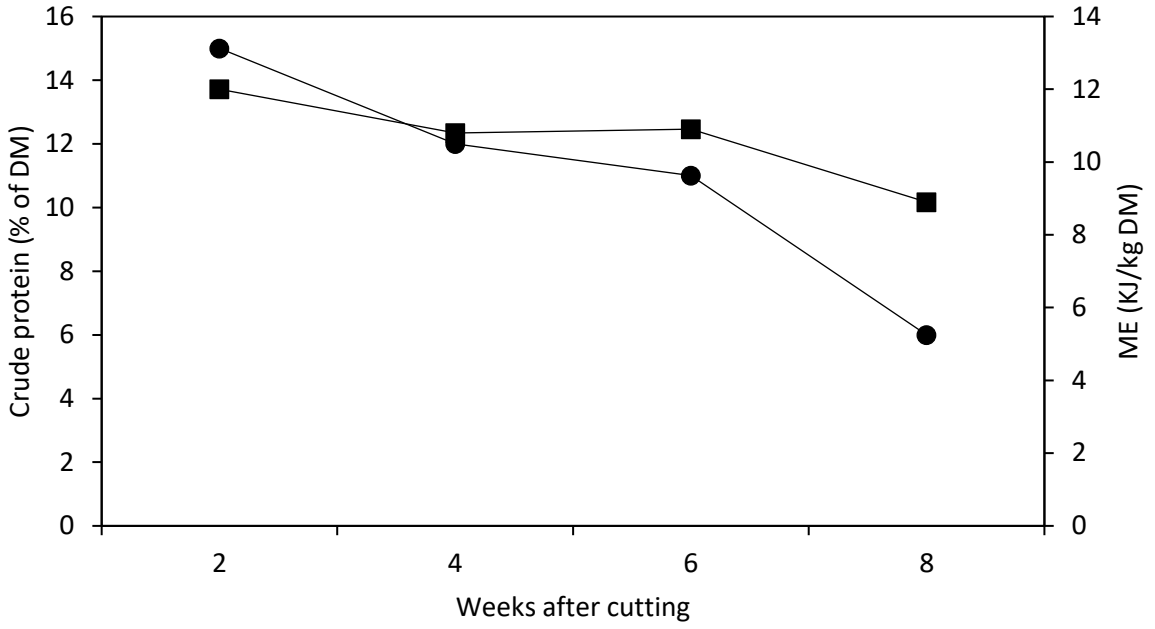

Figure 2.10 Changes in perennial ryegrass (*Lolium perenne*) crude protein (●) and metabolisable energy (ME) (■) over 8 weeks after cutting. Adapted from Waghorn and Clark (2004).

2.3.2 **WSC:CP ratios**

Elevated levels of water soluble carbohydrates (WSC), relative to crude protein (N), have been linked to a reduction in N losses from the urine (Miller *et al.* 2001). This is because WSC provides the microbes in the rumen with an energy source to break down the N into a form which can be absorbed in the rumen for growth and production rather than being lost in the urine (Beever *et al.* 1986; Huntington 1984; Kingston - Smith and Theodorou 2000). In addition, an increase in WSC concentrations have been associated with a reduced N concentration in the herbage potentially leading to lower herbage N intake (Cosgrove *et al.* 2009). Plant breeders in the UK have developed perennial ryegrass cultivars with high concentrations of WSC in the leaf blade (also known as high sugar perennial ryegrasses) to improve the balance of energy to N in the rumen and enhance the supply of microbial protein to the ruminant (Miller *et al.* 2001; Moorby *et al.* 2006). However, there had been considerable debate over the benefits of using high WSC in grasses in New Zealand systems due to climatic differences between the UK and New Zealand (Cosgrove *et al.* 2007; Parsons *et al.* 2004) that may alter the expression of high WSC traits (Edwards *et al.* 2007). In addition, in Europe, diets of dairy cows may be low in N concentration. Thus, increasing WSC concentrations may lead to environmental benefits through the capture of N (Miller *et al.* 2001; Moorby *et al.* 2006). However, in New Zealand, pastures have a higher N concentration through the improved capture of N from legumes and high N fertiliser use. Therefore, large amounts of WSC are needed to offset the imbalance of N (Edwards *et al.* 2007). This is shown in Figure 2.11 where urinary N decreases from 55 g N/100 g N intake to 17 g N/100 g N intake when WSC:CP ratios increase from 0.5 to 2.3. Edwards *et al.* (2007) suggested that a WSC concentration relative to CP of higher than 0.7 was needed to make significant changes in N capture. Considering this,

the ratio of WSC to CP in plants may be important to reducing N losses because of their influence on capturing N in the rumen, ultimately effecting the amount of surplus N converted into the urine or faeces (Holmes *et al.* 2007b; Pichard and Van Soest 1977).

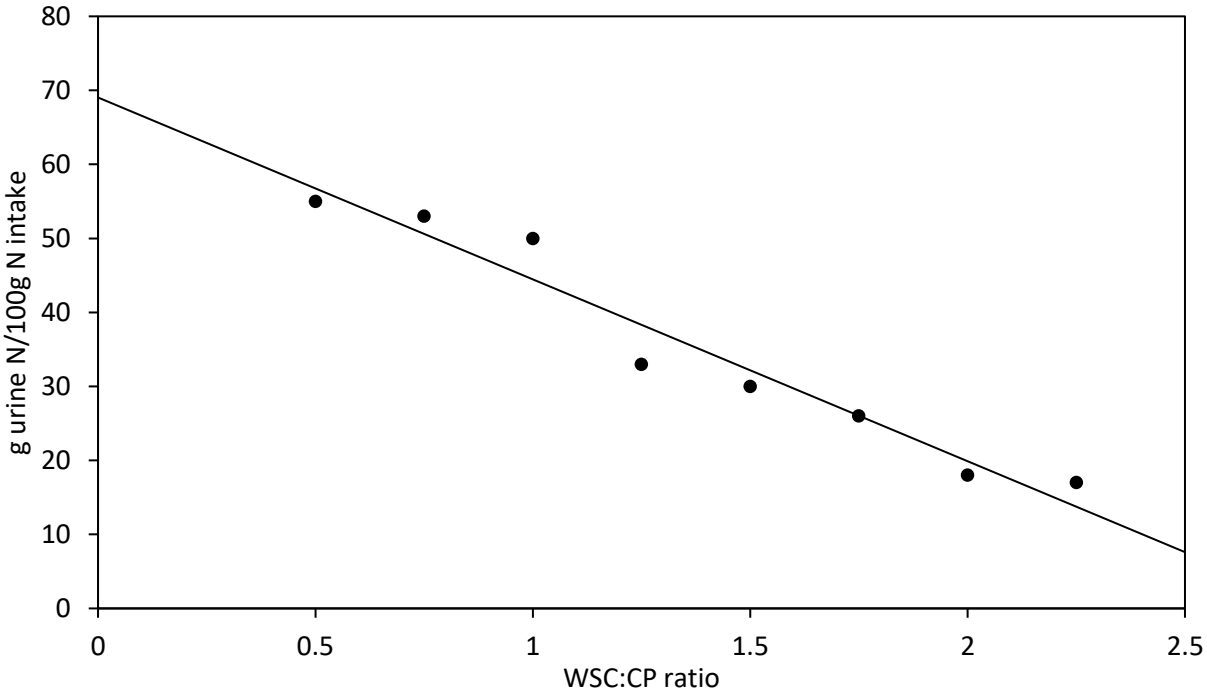

Figure 2.11 Combined data for a range of both UK and Dutch experiments showing a continuum between N utilisation efficiency for urine in dairy cows, in relation to the WSC:CP ratio of the forage component of the diet offered. Adapted from Edwards et al. (2007).

Forage effect

The ratio of WSC:CP has been shown to vary considerably between perennial ryegrass cultivars at different times of the year. For example Bryant *et al.* (2012) showed higher WSC concentrations (20.5 % vs 18.0 %) and lower herbage N concentrations (27.5 % vs 28.5 %) in high sugar perennial ryegrasses (Aberdart), compared to standard perennial ryegrass cultivars (Impact and Grassland Samson). Although these were not large differences in N concentrations, this resulted in significant differences in WSC:CP ratios of 1.01 (standard perennial ryegrass) to 1.19 (high sugar perennial ryegrass). Likewise, Cosgrove et al. (2007) showed during spring, high sugar perennial ryegrass and Italian ryegrass, were 20-40 g/kg DM higher in WSC concentration than standard perennial ryegrass and the WSC:CP ratios were up to 0.3 units higher in the same cultivars. (Table 2.7). However, in autumn the differences were smaller and not significant.

Table 2.7 The concentrations of WSC, CP and the WSC:CP ratio of a standard perennial ryegrass (*Lolium perenne*), a 'high WSC' perennial ryegrass (*Lolium perenne*) and tetraploid Italian ryegrass (*Lolium multiflorum*) during experimental periods in spring 2004 and 2005 and autumn 2006 and 2007. Adapted from Cosgrove et al. (2007).

Season	Cultivar	WSC (% of DM)	CP (% of DM)	WSC:CP ratio
Spring 2004	Standard perennial ryegrass	16.7	22.7	0.7
	High sugar grass	20.0	19.6	1.0
	Italian ryegrass	21.0	20.3	1.0
Spring 2005	Standard perennial ryegrass	19.5	23.5	0.8
	High sugar grass	21.5	23.4	0.9
	Italian ryegrass	21.5	25.5	0.8
Average		20.0	22.5	0.9
Autumn 2006	Standard perennial ryegrass	16.1	25.6	0.6
	High sugar grass	17.0	26.2	0.6
	Italian ryegrass	15.9	28.3	0.6
Autumn 2007	Standard perennial ryegrass	15.0	24.9	0.6
	High sugar grass	15.9	25.5	0.6
	Italian ryegrass	-	-	-
Average		16.7	25.5	0.7

Herbage WSC:CP ratios have also been shown to vary considerably between different forages, at different times of the year. An example of this was in Box *et al.* (2017), where the WSC:CP ratios were considerably lower in plantain in the spring, compared to autumn (6.1 % vs 14.1 %), (Table 2.8). Furthermore, Turner *et al.* (2006) found the amount of WSC per tiller of perennial ryegrass was generally lower compared with that in cocksfoot (September to January) and prairie grass (September, and March to May) (Table 2.9). Thus, these grass forages could be used as an alternative to perennial ryegrass to provide microbial protein more energy to convert N into animal produces rather than urinary N.

Table 2.8 Chemical composition (% of DM) of perennial ryegrass pasture (*Lolium perenne*), plantain (*Plantago lanceolata*) or 50–50 perennial ryegrass pasture–plantain sampled to ground level in autumn 2015 and spring 2016. Adapted from Box *et al.* (2017).

Season	Treatment	WSC (% of DM)	CP (% of DM)	WSC:CP ratio
Autumn 2015	Perennial ryegrass pasture	7.6	23.3	0.3
	50-50 pasture -plantain	9.0	22.2	0.4
	Plantain	10.9	22.3	0.5
Spring 2016	Perennial ryegrass pasture	14.1	14.1	1.0
	50-50 pasture -plantain	10.7	17.8	0.6
	Plantain	6.1	19.5	0.3

Table 2.9 Mean amount of water soluble carbohydrates per tiller (% of DM) of prairie grass (*Bromas willdenowii*), cocksfoot (*Dactylis glomerata*) and perennial ryegrass (*Lolium perenne*) defoliated at bi-monthly intervals. Adapted from Turner *et al.* (2006).

Month	Prairie grass	Cocksfoot	Perennial ryegrass
	----------% of DM----------		
September	33	13.9	7
November	10.3	21	9
January	8.2	15.3	8.2
March	16.5	7.9	8.3
May	12.9	5.8	8.4
Average	16.18	12.78	8.18

However, it has been found herbage WSC concentration is affected by diurnal changes, herbage WSC concentrations are lower in the morning than in the afternoon (Bryant *et al.* 2012; Ciavarella *et al.* 2000). For example, in Bryant *et al.* (2012), averaged over the N fertiliser rates and cultivars, and at the third leaf stage, WSC concentrations were lower in the AM treatment (18.8 % of DM) compared to the PM treatment (21.4 % of DM). Differences in sampling regime may affect results, thus it is difficult to compare the WSC:CP ratios in forages in previous experiments because time of day has such a large effect. Consequently, an experiment comparing the WSC:CP ratios of a range of forages in a controlled environment is needed to identify whether some have lower WSC:CP ratios than others thus, effecting N losses in animals.

N fertiliser effect

Nitrogen fertiliser rates have been shown to affect the WSC:CP ratios in a variety of grasses by increasing the amount of N in the herbage (Waite 1958). This was shown in Table 2.10 where as fertiliser increased from 0 to 448 kg N/ha, WSC:CP ratio decreased from 4.0 to 1.4 in the slow growing ryegrass (S23), 2.7 to 0.7 in fast growing ryegrass (S24), 1.8 to 0.6 in the fescue, 2.3 to 0.6 in the timothy and 1.4 to 0.3 in the cocksfoot grasses. In addition Bryant *et al.* (2012) showed two out of three cultivars had elevated herbage N and depressed WSC concentrations after increased rates of 25 kg N/ha were applied (Figure 2.12).

Table 2.10 Effect of N fertiliser on the WSC:CP ratio of various grass species. Adapted from Waite (1958).

Grass	Scientific name	Fertiliser rate (kg/ha)		
		0	**224**	**448**
Ryegrass S23*	*Lolium perenne*	4.0	3.4	1.4
Ryegrass S24**	Lolium perenne	2.7	2.7	0.7
Fescue	*Festuca arundinacea*	1.8	1.2	0.6
Timothy	*Phleum pratense*	2.3	1.2	0.6
Cocksfoot	*Dactylis glomerata*	1.4	0.5	0.3

* slow growing ryegrass
** fast growing ryegrass

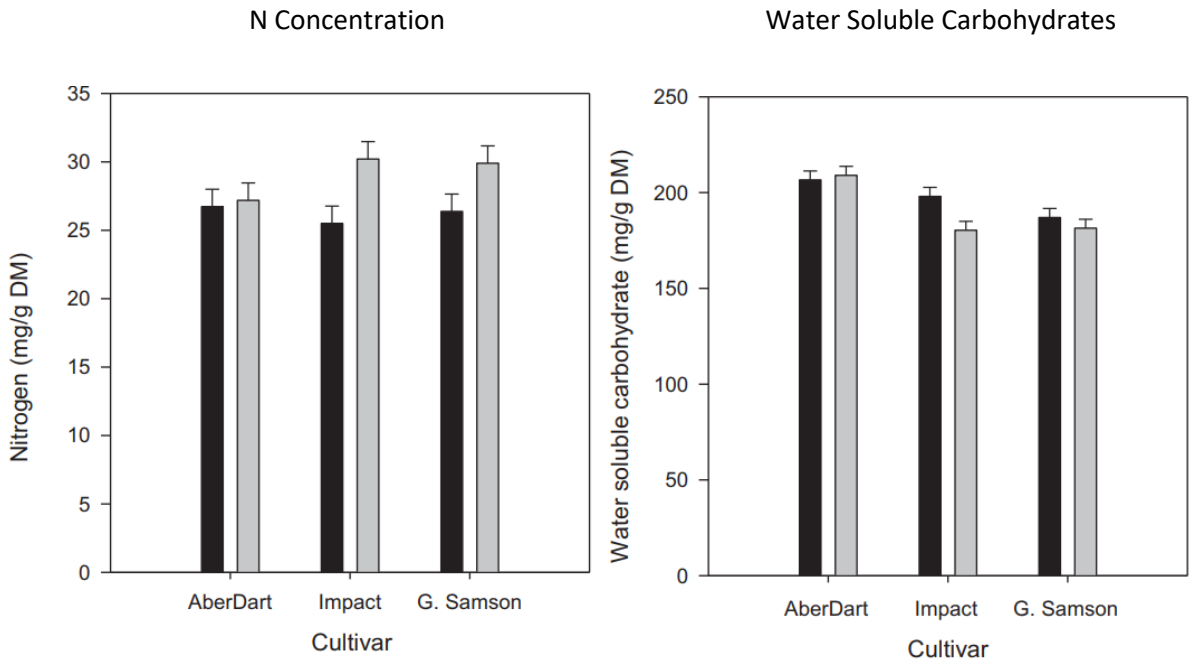

Figure 2.12 Nitrogen and water soluble carbohydrate concentration of three perennial ryegrass (*Lolium perenne*) cultivars following no fertiliser N application (black columns) or an application of 25 kg N/ha (grey columns). Adapted from Bryant *et al.* (2012).

In addition, Loaiza *et al.* (2016) showed a similar finding with WSC:CP ratio decreasing as N fertiliser was applied; from 0.60 at 0 kg N fertiliser to 0.32 at 450 kg N fertiliser in spring, and from 0.94 at 0 kg N fertiliser to 0.47 at 450 kg N fertiliser in autumn (Table 2.11).

Table 2.11 Effects of N fertilisation rate (kg N/ha/yr) on nutritive quality of perennial ryegrass (*Lolium perenne*) during early spring and autumn. Adapted from Loaiza *et al.* (2016).

	0	**75**	**150**	**300**	**450**
Early spring					
CP (g/kg DM)	203	221	231	255	275
WSC (g/kg DM)	118	105	107	97	87
WSC: CP	0.60	0.48	0.47	0.39	0.32
Autumn					
CP (g/kg DM)	178	183	200	224	250
WSC (g/kg DM)	162	164	146	132	113
WSC: CP	0.94	0.96	0.76	0.64	0.47

These results indicate high N fertiliser inputs present an increased risk of N losses from the system due to the reduction in energy (WSC) needed for the microbes to break down the N for absorption. Thus, reducing the amount of N fertiliser could be a mitigation strategy to increase the WSC:CP ratios and therefore possibly reduce the amount of nitrate leaching through providing the rumen microbes with more energy for converting N into animal products.

Regrowth interval effect

Plant WSC concentrations are also affected by timing of grazing. This was shown in Fulkerson and Slack (1994) where, as regrowth interval increased from 0 to 4 leaves, WSC concentrations in perennial ryegrass increase from 2.5 % of DM to 14 % of DM (Figure 2.13). This was due to plant replenishment of reserves after harvest (Fulkerson and Slack 1994; Rawnsley *et al.* 2002). WSC have also been shown to dilute the N concentration in the pasture meaning there is lower proportion of N in the diet to be lost in the urine (Cosgrove *et al.* 2009). Consequently, this equates to a higher ratio of WSC to CP later in the regrowth period.

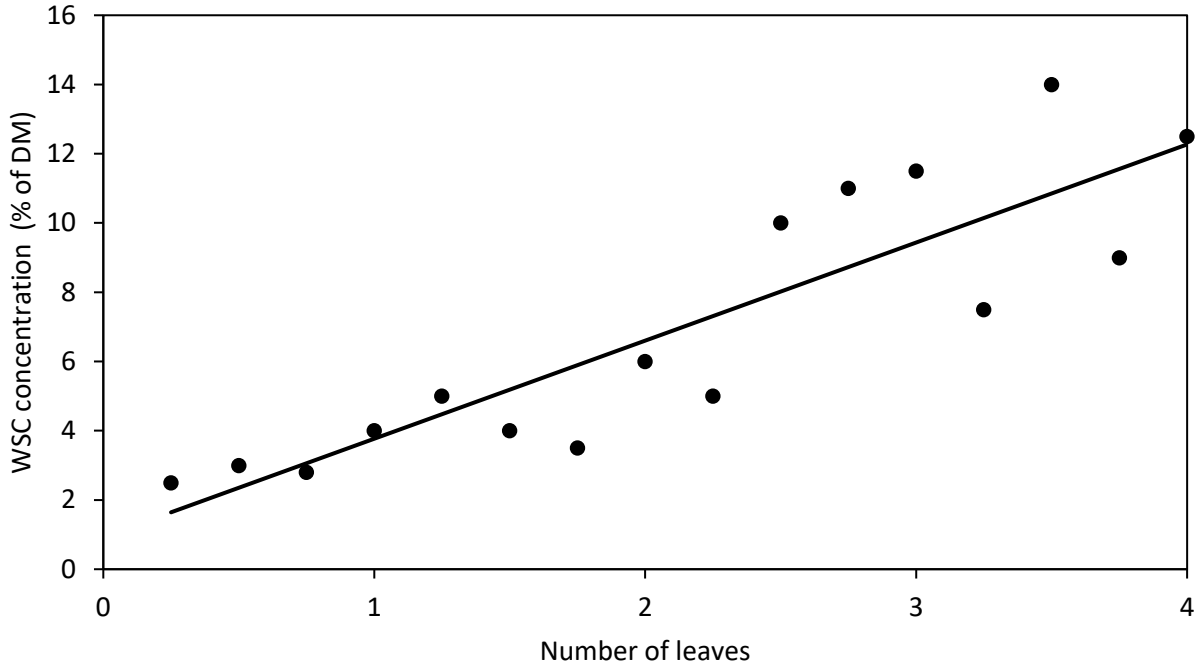

Figure 2.13 Changes in WSC concentrations of perennial ryegrass (*Lolium perenne*) leaves during regrowth, with regrowth time expressed in leaves/tiller (adapted from Fulkerson and Slack (1994)).

In addition, WSC:CP ratios were also found in perennial ryegrass to be lower earlier in the growth stage (Loaiza *et al.* 2016). This is shown in Figure 2.14 where, in early spring, late spring and autumn the two leaf stage plants contained lower WSC:CP ratio (0.7 to 0.3) than the three leaf stage plants (WSC:CP ratio of up to 1.6).

Overall, delaying grazing to later in the regrowth period could provide the microbes with more energy to break down N for absorption for growth and development of the animal.

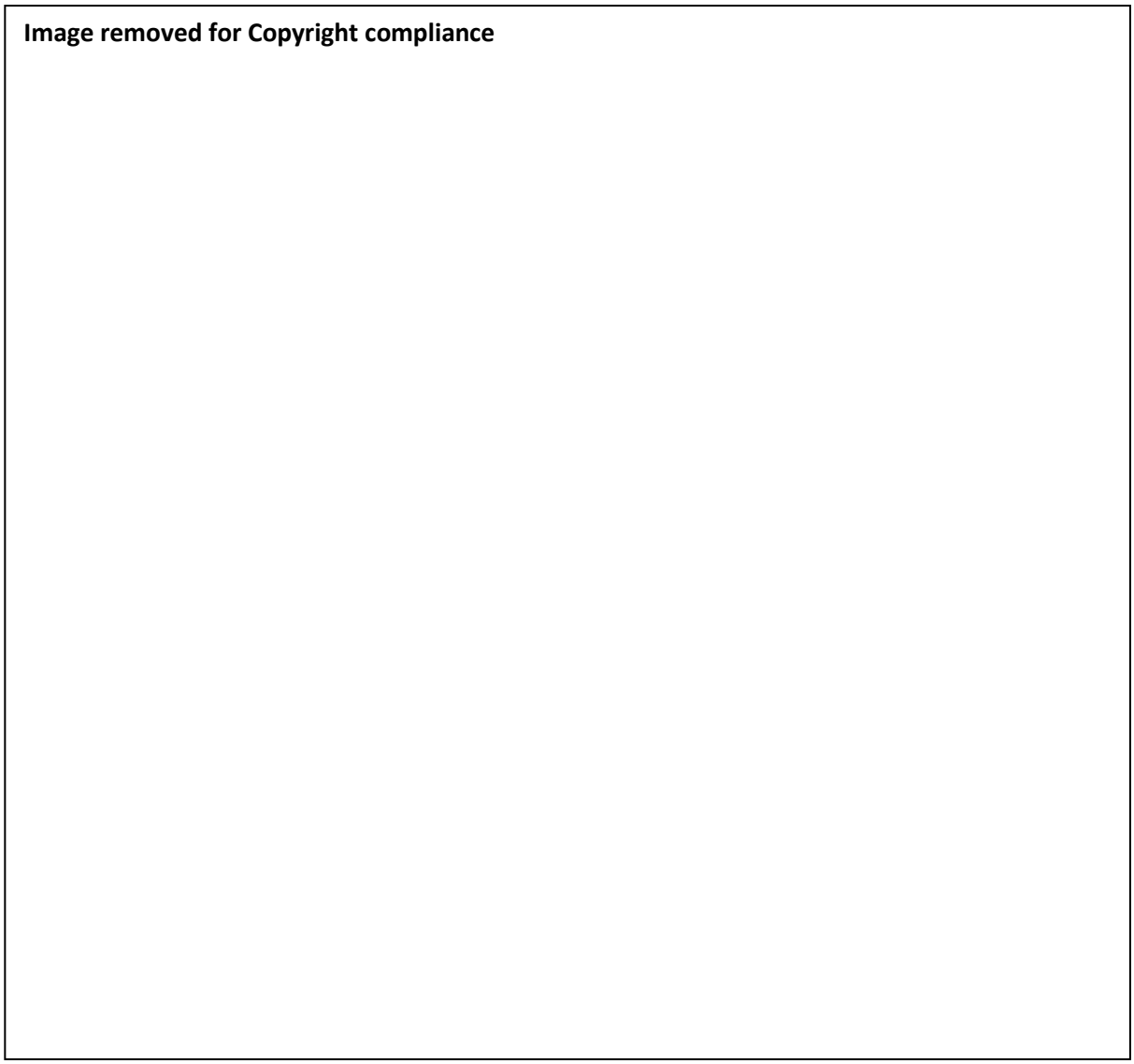

Figure 2.14 Changes in water-soluble carbohydrate-to-protein ratio (WSC:CP) in perennial ryegrass (*Lolium perenne*) defoliated either at two-leaf or three-leaf stages during early spring, autumn, late spring and summer in response to N fertilization rate. Verticals bars represent the standard error of the mean (n = 3). From Loaiza *et al.* (2016).

2.3.3 N solubility

While the amount of dietary N has implications on the total surplus N in ruminant diets, there is large variation in how dietary N is utilised and partitioned to product (Holmes *et al.* 2007b; Pichard and Van Soest 1977). For example, high N diets, which contain soluble N can cause an inefficiency of plant N utilisation by the microbes in the rumen (Pacheco and Waghorn 2008). This is because the soluble N is available immediately for converting into microbial protein, however the rate microbes process the N is too slow. Therefore, in the absence of an adequate energy source, the animal converts the dietary N into ammonia and it is absorbed through the rumen wall, converted into urea in the liver, and mainly excreted in the urine (Castillo *et al.* 2001; Pacheco and Waghorn 2008). Consequently, less soluble proteins are more desirable for reducing N losses. This is because less soluble forms of N are slower to degrade in the rumen and may escape rumen degradation altogether if fractional outflow rates are

great (Stout *et al.* 1997). This results in better utilisation of the plant within the animal because the rumen microbes can process the N needed for animal production and growth. With this in mind, when investigating alternative pasture forages to reduce N losses, some forages may have similar herbage N concentrations but have different N solubility and therefore different efficiency rates to utilise N.

Forage effect

Variation in plant solubility has been shown within grass and legume forages (Brown and Pitman 1991; Bryant *et al.* 2012; Da Silva *et al.* 2014; Elizalde *et al.* 1999; Kirchhof *et al.* 2010; Sanderson and Wedin 1989) suggesting some forages and cultivars are more suitable for capturing N in the rumen than others. For example, Bryant *et al.* (2012) showed the soluble N fraction A was significantly higher in the high sugar perennial ryegrass Aberdart (0.17 % of N) than the other standard perennial ryegrass cultivars (0.155 % of total N) and soluble N fraction B was significantly lower (P=0.04) in the high sugar perennial ryegrass Aberdart (0.43 % of N) than the other standard perennial ryegrass cultivars (0.445 % of total N) (Table 2.12).

Table 2.12 Soluble (fraction A and B1) and insoluble (B2 and nutrient detergent insoluble N (NDIN)) N fractions of perennial ryegrass (*Lolium perenne*) cultivars. Adapted from Bryant et al. (2012).

Cultivar	A	B1	B2	NDIN
	----------% of total N----------			
Aberdart	0.17	0.43	0.3	0.1
Impact	0.15	0.45	0.32	0.08
Samson	0.16	0.44	0.32	0.08
F pr.	**0.02**	**0.04**	NS	NS

F pr., significance; NS, not significant.

Another example where there is variation among forages is Kirchhof *et al.* (2010) who found soluble N fractions (A and B1) were lowest in the birdsfoot trefoil (319 g/kg CP) and highest in the lucerne and white clover (529 and 525 g/kg CP, respectively) (Table 2.13). Therefore, in this instance, birdsfoot trefoil was more suited to increase N use efficiency in the rumen and reduce N in the urine because it has a larger proportion of insoluble protein which the microbes can utilise better. This may be due to condensed tannins which have been found to bind to proteins and increase the absorption of protein in the small intestines rather than the rumen (Min *et al.* 2003).

Table 2.13 Crude protein concentration and N fractions of six legume forages at vegetative or early flowering stage. Adapted from Kirchhof *et al.* (2010).

Forage	Scientific name	Crude protein (CP)	A	B1	B2	B3	C
		g/kg DM	----------g/kg CP----------				
White clover	*Trifolium repens*	210	232	293	443	12	21
Red clover	*Trifolium pratense*	145	196	296	443	39	27
Lucerne	*Medicago sativa*	170	176	353	428	14	29
Birdsfoot trefoil	*Lotus corniculatus*	170	169	150	637	16	28
Kura clover	*Trifolium ambiguum M.B.*	170	221	298	456	8	18

Interestingly, Da Silva *et al.* (2014) showed the lucerne and grass mixture were similar in total N (24.9 and 27.3 g/kg DM) and subsequent N fractions (776 and 781 g/kg total N as soluble N and 178 and 175 g/kg total N as insoluble N) (Table 2.14). This was not expected and may be due to the time of harvesting the lucerne. As previously mentioned, a longer regrowth interval decreases the concentration of N in the plant. Da Silva *et al.* (2014) showed the lucerne and grass mix were more suited to reduce N losses because they have a smaller proportion of highly soluble proteins and larger amount of insoluble protein which results in better utilisation of the plant N in the rumen.

Table 2.14 Total N and subsequent N fractions of lucerne (*Medicago sativa*), birdsfoot trefoil (*Lotus corniculatus*) and a grass mixture. Adapted from Da Silva *et al.* (2014).

	Total N	Non-protein N	Rapidly degraded protein	Intermediate degraded protein	Insoluble protein
	g/kg DM	----------g/kg total N----------			
Lucerne	24.9	225	145	408	178
Birdsfoot trefoil	30.1	236	129	411	169
Grass mix*	27.3	225	146	410	175

* timothy (*Phleum pratense*), meadow fescue (*Festuca pratensis*), kentucky bluegrass (*Poa pratensis*).

Furthermore, Brown and Pitman (1991) showed legumes were higher in total N with values of 39.2 g/kg DM (*Aeschynomene americana*) and 30.4 g/kg DM (*Indigolera hirsuta*) for legumes and values of 13.3 g/kg DM (H*ermathria altissima*) and 6.8 g/kg DM (*Paspalum notratum*) for tropical grasses (Table 2.15). However, legumes were lower (302 g/kg N) than the tropical grass species (371 g/kg N) in soluble N and higher in insoluble N (699 g/kg N) than the tropical grass species (625 g/kg N). This result suggests in tropical conditions, legumes are more suited to reduce N losses because the microbes in the rumen can utilise the plant N more efficiently for conversion into animal products.

Table 2.15 Total N and subsequent N fractions of two tropical grass species and two legumes species. Adapted from Brown and Pitman (1991).

	Total N	Soluble N (A + B1)	Insoluble, potentially degradable (B2+B3)	ADIN (C)
	g/kg DM	----------g/kg N----------		
Tropical grass (H*ermathria altissima*)	13.3	352	564	83
Tropical grass (*Paspalum notatum)*	6.8	390	529	74
Legume *(Aeschynomene americana)*	39.2	324	640	36
Legume *(Indigolera hirsuta*)	30.4	279	681	40

Finally, Sanderson and Wedin (1989) showed, after 4 weeks, lucerne was lowest in neutral detergent insoluble N (NDIN) (61.0 g/kg total N) and acid detergent insoluble N (ADIN) (17.0 g/kg total N) (Table 2.16). This is likely due to the lower amount of NDF and ADF in the lucerne and higher quality of the forage. The NDIN and ADIN was highest in the timothy species (214.5 and 39.6 g/kg total N). This was because of the large amount of stem containing ADF and NDF. Overall, it was shown that alternative

pasture forages can be different in their N solubility and therefore different in their ability to provide animals with high or low utilisation of N in the rumen.

Table 2.16 Leaf concentrations of N in neutral detergent fibre (NDIN) and acid detergent fibre (ADIN), after 4 weeks of growth, expressed on a total nitrogen basis (g/kg total N). Adapted from Sanderson and Wedin (1989)

Forage	Scientific name	NDIN	ADIN
		----------g/kg total N----------	
Lucerne	*Medicago sativa*	61.0	17.0
Red clover	*Trifolium pratense*	116.0	26.0
Timothy	*Phleum pratense*	214.5	39.5
Bromegrass	*Bromus madritensis*	133.0	26.5

N fertiliser effect

The effects of N fertiliser have been shown to affect plant N solubility in perennial ryegrass and therefore, change the N cycling within the plant. Higher N fertiliser rates increase the proportion of soluble N, which contains non-protein N (NPN) such as peptides, free amino acids (AA), nitrate and nitrite. This was shown in Goswami and Willcox (1969) (Table 2.17) where after high N applications of up to 500 kg N/ha were applied, plants continued to take up N from the soil in luxury amounts as the form of NPN or soluble N (up to 9.4 % of total N as nitrate and nitrite and 15.0 % of total N as free AAs) which is unfavourable to reduce the rate of N converted into urea and urinary N.

Table 2.17 Nitrogen constituents in perennial ryegrass (*Lolium perenne*) receiving 60, 120, 250 and 500 kg N/ha averaged from cuts at vegetative, heading and flowering stages of growth. Adapted from Goswami and Willcox (1969).

	N application (kg/ha)			
	60	**120**	**250**	**500**
Total N (g/100g DM)	1.53	1.89	2.69	3.73
Protein (% of total N)	72	66.7	65.3	55.2
Peptide (% of total N)	9.1	8.5	6.3	7.2
Free AA (% of total N)	10.4	11.1	11.5	15.0
Nitrate and nitrite (% of total N)	2.6	3.2	6.3	9.4
Other organic N (% of total N)	2.6	3.2	8.9	10.7

AA: amino acids

However, most experiments indicated that N solubility is not affected by N application rate (Bryant *et al.* 2012; Hoekstra *et al.* 2008; Ledgard *et al.* 1990). This was shown in Ledgard *et al.* (1990) where soluble N and insoluble N was similar in both the 0 kg N/ha fertiliser treatment and 50 kg N/ha fertiliser treatment (soluble N 307 and 309 g/kg N , insoluble N 693 and 691 g/kg N), averaged over the regrowth period (Table 2.18).

Table 2.18 Proportion of soluble N and insoluble N two different N fertiliser rates 33 days after harvest of perennial ryegrass (*Lolium perenne*). Adapted from Ledgard *et al.* (1990).

	Soluble N		Insoluble N	
Regrowth (days)	**High N**	**Low N**	**High N**	**Low N**
	----------g/kg N----------			
0	270	270	730	730
5	378	324	622	676
10	300	289	700	711
15	302	278	698	722
20	270	314	730	686
33	323	379	677	621
Average	307	309	693	691

Similar results were found in Bryant *et al.* (2012) where there were no significant differences between the soluble and insoluble N fractions between the two N fertiliser rates (0 kg N/ha and 25 kg N/ha) (Figure 2.15). Overall, the N fractions in the herbage remain relatively constant as a proportion of total N, independently of N application rate.

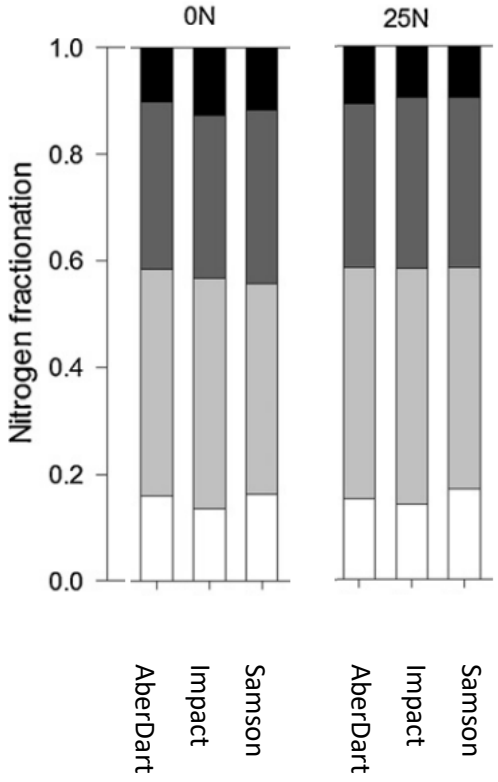

Figure 2.15 Fractionation of total N into: A (white), B1 (light grey), B2 (dark grey) and NDIN (black) of three perennial ryegrass (*Lolium perenne*) cultivars at 3 leaf appearance under two fertiliser regimes (0 and 25 kg N/ha) in early spring. Adapted from Bryant *et al.* (2012).

Regrowth interval effect

The effect of regrowth interval on N solubility is apparent. It has been shown as regrowth interval increases in perennial ryegrass forages, soluble N concentration in the herbage increases (Amrane and Michalet-Doreau 1993; Bryant *et al.* 2012). This was shown in Bryant et al. (2012) where soluble N

increased from 50% of total N in the plant at the 2 leaf stage to 57% of total N in the plant at the 4 leaf stage (Figure 2.16).

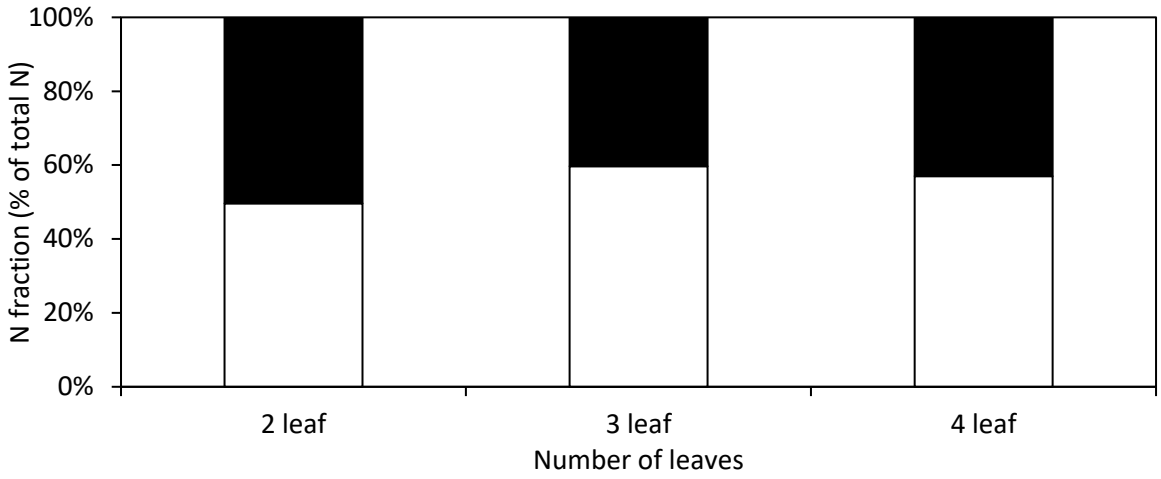

Figure 2.16 Percentage of soluble N (white) and insoluble N (black) in perennial ryegrass (*Lolum perenne*) over regrowth interval as expressed as number of leaves. Adapted from Bryant et al. (2012).

Likewise, Loaiza *et al.* (2016) showed as regrowth of perennial ryegrass swards increased from two leaves to three leaves, soluble N fractions A and B1 increases from 339 g/kg CP to 408 g/kg CP (Table 2.19). Consequently, insoluble N fractions decreased as regrowth increased from 661 to 592 g/kg CP (total of B2, B3 and C). These results were likely due to older tissue converting insoluble storage N back into soluble N to be utilised for new growth (Hörtensteiner and Feller 2002) and an increase in stem material which contains a higher amount of soluble N in the form of NPN (Hoekstra *et al.* 2007). As a result, a longer regrowth interval may cause a higher conversion of N to urea in the rumen, triggering lower utilisation of N in the animal for growth and production, and higher urinary N.

Table 2.19 Effects of defoliation frequency (two or three leaf stage) and season on crude protein (CP) concentration and crude protein fractions as a proportion of total CP of perennial ryegrass (*Lolium perenne*). Adapted from Loaiza *et al.* (2016).

		Crude protein	A + B1	B2	B3	C
		g/kg DM	----------g/kg CP----------			
Early spring	Two leaf	260	464	488	42	5
	Three leaf	214	456	482	52	9
Late spring	Two leaf	181	275	652	60	12
	Three leaf	128	324	552	105	18
Summer	Two leaf	152	303	518	161	19
	Three leaf	133	414	400	155	30
Autumn	Two leaf	231	312	549	127	11
	Three leaf	182	437	448	99	17
Average	Two leaf	206	339	552	98	12
	Three leaf	164	408	471	103	19

Sanderson and Wedin (1989) also investigated the effects of insoluble fractions NDIN and ADIN over the regrowth interval. Results showed that NDIN increased from 95.6 g/kg total N to 131.1 g/kg total N, averaged over the four species (Table 2.20). In addition, ADIN increased from 11.4 g/kg total N to 27.3 g./kg total N. This result is likely due to a larger amount of stem and dead material which contains higher amounts of ADF and NDF. Overall, it is unclear whether regrowth interval increases or decreases the utilisation of plant N in the animal rumen.

Table 2.20 Leaf concentrations of N in neutral detergent fibre (NDIN) and acid detergent fibre (ADIN), expressed on a total nitrogen basis (g/kg total N). Adapted from Sanderson and Wedin (1989).

Forage	Scientific name	Harvest number			
		1	2	3	4
NDIN		----------g/kg total N----------			
Lucerne	*Medicago sativa*	36.5	36.5	42.0	61.0
Red Clover	*Trifolium pratense*	157.0	140.0	116.0	116.0
Timothy	*Phleum pratense*	102.0	126.5	163.5	214.5
Bromegrass	*Bromus madritensis*	87.0	91.0	124.0	133.0
Average		95.6	98.5	111.4	131.1
ADIN					
Lucerne	*Medicago sativa*	13.0	14.0	15.5	17.0
Clover	*Trifolium pratense*	8.0	12.0	18.0	26.0
Timothy	*Phleum pratense*	14.0	18.5	29.5	39.5
Bromegrass	*Bromus madritensis*	10.5	14.0	19.5	26.5
Average		11.4	14.6	20.6	27.3

2.4 Conclusions

The main conclusions drawn from this literature review are:

- Nitrate leaching has become a major concern for the environmental sustainability of dairy systems in New Zealand. This is caused mainly from urine patches where larger amounts of nitrate is present than needed for the plant's growth.

- Mitigation options are urgently required to reduce nitrate losses whilst maintaining farm production and profit. One way to achieve this is to reduce the amount of excess N in the farm system to decrease N losses.

- Strategies proposed to reduce N losses in the farm system include increasing DM responses to N fertiliser, lowering herbage N concentrations and N solubility, increasing the WSC:CP ratios of herbage grazed by animals.

- This can be done through extending the regrowth interval, decreasing N fertiliser application in perennial ryegrass –white clover pastures. However, little is known about alternate pasture species.

- Therefore, an opportunity has emerged to compare a range of alternate pastures with different functional groups to identify whether some plants possess more desirable traits than others to reduce nitrate leaching, making them environmentally friendly species, without impeding farm production and profitability. Consequently, this is the focus of this PhD research.

Chapter 3

Herbage dry matter yield and nitrogen concentration of grass, legume and herb forages grown at different nitrogen fertiliser rates under irrigation in Canterbury

3.1 Introduction

Regulations that place a limit on the amount of nitrate leaching from agricultural land are currently being developed by Regional Councils throughout New Zealand (Ministry for the Environment 2014). These regulations may require substantial reductions in nitrate leaching from current typical dairy-farm levels and approaches are sought to achieve these reductions, while sustaining or improving profitability (Bryant *et al.* 2007).

The urine patch is the primary source of leached nitrate in pasture-based dairy systems (Di and Cameron 2002c). The main factor influencing the amount of nitrogen (N) excreted in urine is the amount of N consumed by animals relative to the demands of production, maintenance and body tissue retention (Kebreab *et al.* 2001). Therefore, a logical pathway to controlling N surplus in the animal (the amount of N ingested minus amount required) is to manipulate the N concentration in the feeds they are eating, provided intakes are the same. One approach is to identify forages that result in a lower herbage N intake per kg dry matter (DM) consumed. However, while N fertiliser rate experiments have improved our knowledge of the relationships between soil N fertiliser, herbage DM yield and N composition of herbage in some grasses (perennial ryegrass (Hill *et al.* 2005) and cocksfoot (Mills *et al.* 2009)), less information is available for alternative grasses (e.g. tall fescue, Italian ryegrass, prairie grass, high sugar perennial ryegrass) herbs (e.g. chicory and plantain) and legume forages (e.g. lucerne, red clover and white clover). This information is necessary to identify candidate pasture forages for future forage systems that lead to lower nitrate leaching losses whilst maintaining high herbage DM yields and farm profitability.

Therefore, the objective of this experiment was to quantify the effect of N fertiliser rate on herbage DM yield and N concentration, in plantain and chicory, lucerne, red clover, white clover, prairie grass, Italian ryegrass, tall fescue, tetraploid perennial ryegrass, diploid perennial ryegrass, high sugar perennial ryegrass and cocksfoot at optimum defoliation time over a two-year period.

3.2 Materials and methods

3.2.1 Experimental site and design

The experiment was conducted from 1 December 2014 to 30 November 2016 at the Lincoln University Research Dairy Farm (LURDF), Canterbury, New Zealand (43°64'S, 172°46'E). The soil type was a free-draining Templeton fine sandy loam (Immature Pallic soil; (Hewitt 2010) Plate 3.1).

Plate 3.1 Soil profile of Templeton fine sandy loam on LURDF, Canterbury NZ.

The experiment consisted of four replicates of a split-plot factorial design, with 12 forages as the main plot treatments and six annual N fertiliser rates as subplot treatments (Plate 3.2). The 12 forages and their sowing rates are shown in Table 3.1 and the six annual N fertiliser rates are shown in Table 3.2. The main plots were 3 metres (m) × 12.6 m, and subplots 3 m × 2.1 m. All forages were sown as pure swards (Plate 3.2).

Plate 3.2 Experimental area with forages sown as pure swards on LURDF, Canterbury NZ.

Table 3.1 Forages sown and their functional group, scientific name, cultivar and sowing rate.

Pasture	Functional group	Scientific name	Cultivar	Sowing rate (kg/ha)
Diploid PRG – late season flowering	Grass	*Lolium perenne*	One-50 (AR37*)	20
Tetraploid PRG – late season flowering	Grass	*Lolium perenne*	Base (AR37*)	25
HSG - late season flowering diploid	Grass	*Lolium perenne*	AberMagic (AR1*)	18
Tall fescue	Grass	*Festuca arundinacea*	Easton (MaxP*)	25
Italian RG - diploid	Grass	*Lolium multiflorum*	Tabu	25
Cocksfoot	Grass	*Dactylis glomerata*	Savvy	8
Prairie grass	Grass	*Bromas willdenowii*	Atom	25
Chicory	Herb	*Cichorium intybus*	Choice	8
Plantain	Herb	*Plantago lanceolata*	Tonic	10
White clover	Legume	*Trifolium repens*	Kopu 2	5
Red clover	Legume	*Trifolium pratense*	Sensation	10
Lucerne	Legume	*Medicago sativa*	Torlesse (Superstrike**)	14

Diploid PRG, diploid perennial ryegrass; Tetraploid PRG, Tetraploid perennial ryegrass; HSG, high sugar perennial ryegrass; Italian RG, Italian ryegrass.
* endophyte
** seed coating

Table 3.2 Annual N fertiliser rates applied to plots.

Grasses	Herbs	Legumes
----------Kg N/ha/year----------		
0	0	0
45	45	39
90	90	78
180	180	156
315	315	272
450	450	389

3.2.2 **Management**

The site was sprayed with glyphosate, cultivated with a Duncan Contoura with rear crumbler, power-harrowed using a LELY Power Harrow (2.5 m width) and rolled with a Cambridge Roller in March 2014. The plots were sown with a Flexiseeder 14 row plot drill (width 2.1 m) on 20 March 2014. Soil nutrient sampling was conducted before the experiment in May 2011 with a soil corer to a depth of 75 millimetres (mm); results showed pH = 6.1 (soil : water ratio, 1 : 2), Olsen phosphorus (P) = 26 mg/L (Olsen *et al.* 1954) sulphate-sulphur (sulphate-S) = 5 mg/kg (Watkinson and Kear 1994) and potassium (K) = 0.23 meq/100 g (Rayment and Higginson 1992) (Table 3.3). Based on this, plots were fertilised as shown in Table 3.4, to ensure that these nutrients were not limiting to the pasture forage under investigation. Due to the harvest method (cut and carry), higher levels and more frequent applications of potassium fertiliser was applied to the lucerne plots to keep up with greater nutrient demands

(Harris *et al.* 1966; Metson 1974). Herbicide 'T Max' (40 mL/10 L water) was applied to the grass and plantain plots regularly to remove dicotyledon forages, particularly white clover and dock (Table 3.5). Herbicide 'Gallant' (5 ml/10 L water) with surfactant 'Uptake' (25 ml/10 L water) was applied to legumes and herb plots regularly remove grass forages (Table 3.5). An application of herbicide 'Headstart' (10 ml/10 L water) was also applied to legumes and chicory in early 2016 to remove dicotyledon forages.

Table 3.3 Soil test results throughout the 2 year experiment. Plots were sampled at 7 cm depth and sent away to be analysed by Hills Laboratories.

Soil test date	pH	Olsen P	Sulphate super	K	Ca	Mg	Na	CEC
		(mg/L)	(mg/kg)	----------(me/100g)----------				
May 2011	6.1	26	5	0.23	8.1	0.48	0.15	14
Dec 2014	6.1	21	13	0.28	7.8	0.47	0.17	14
Aug 2015	6.1	22	NA	0.59	7.4	0.57	0.15	14
May 2016	6.5	26	4	0.23	9.7	0.44	0.13	14
Dec 2016	6.5	18	3	0.19	10.3	0.45	0.16	13

Table 3.4 Total nutrients applied to each forage.

Date	Forage	Total nutrients applied (kg/ha)			
		P	K	S	Lime
Mar 2014	All forages	12.8	20	32.8	0
Oct 2014	All forages	12.8	20	32.8	2000
Dec 2014	Lucerne	28	75	72	2000
Sep 2015	Lucerne	45	250	55	0

Table 3.5 Herbicide applications to each forage.

Date	Forage type	Herbicide	Active ingredient	Rate
Dec 2014	Legumes and herbs	Gallant*	haloxyfop-P-methyl	5 ml/ 10 L water
Jan 2015	Legumes and herbs	Gallant*	haloxyfop-P-methyl	5 ml/ 10 L water
Jan 2015	Grass and plantain	T Max	aminopyralid	40 ml/10 L water
Feb 2015	Legumes and herbs	Gallant*	haloxyfop-P-methyl	5 ml/ 10 L water
May 2015	Legumes and herbs	Gallant*	haloxyfop-P-methyl	5 ml/ 10 L water
May 2015	Grass and plantain	T Max	aminopyralid	40 ml/10 L water
Jan 2016	Grass and plantain	T Max	aminopyralid	40 ml/10 L water
Jan 2016	Chicory	Gallant*	haloxyfop-P-methyl	5 ml/ 10 L water
Jan 2016	Legumes and chicory	Headstart	flumetsulam	10 ml/10 L water
Feb 2016	Legumes and herbs	Gallant*	haloxyfop-P-methyl	5 ml/ 10 L water
Oct 2016	Grass and plantain	T Max	aminopyralid	40 ml/10 L water

* Gallant was applied with surfactant Uptake at rate of 25ml/10 L water.

The site was irrigated with a self-travelling Roto Rainer irrigator between October and March each year, with 20–30 mm water applied per week (total 550 mm). Plots were allowed to establish in the absence of harvests until spring 2014. They were then cut and fertilised by hand at the beginning of October 2014 and November 2014 before the experiment began. The start of the experiment was 1

December 2014 and results were calculated on a seasonal basis (summer, autumn, winter/early spring, late spring) using daily growth rates. The period for each season is shown in Table 3.4. The timing of harvest was determined using best practice methods for maximising herbage DM yields and persistence in the three different functional groups, namely, grasses, legumes and herbs. For grasses this was at the third leaf stage (Donaghy and Fulkerson 1998), for legumes it was around 35 days of regrowth (Moot *et al.* 2003) and for herbs this was once plants reach 250 mm in height (Lee *et al.* 2015). Grasses and herbs were harvested at 32, 26 and 30 day intervals in spring, summer and autumn respectively. Legumes were harvested at 41, 35 and 41 day intervals in spring, summer and autumn respectively. Due to low soil temperatures and slow growth rates, no plots were harvested in June and July. The number of harvests and seasonal distribution based on forage type are shown in

Table 3.6.

Herbage was harvested and removed from each plot with a Walker MC GHS (20 HP, 14.9 kW) ride on rotary lawnmower, with the cutting height set to 4 cm for all forages (Plate 3.3). Before the experiment began, the planned N fertiliser rates were 0, 50, 100, 200, 350 and 500 kg N/ha/year. It was estimated that 9 (legumes) or 10 (grasses and herbs) harvests would occur and fertiliser application rates were calculated on this basis. A lower number of harvests in legumes was proposed because of slower growth rates. However, due to best practice methods, all plots were harvested fewer times than expected and instead, nine harvests for grasses and herbs and seven harvests for legumes occurred each year (1 December to 30 November). This led to lower rates of N applied than proposed and differences in rates of N fertiliser applied between legumes and grasses and herbs. The application rates for each forage and N rate are shown in Table 3.7. Nitrogen fertiliser was applied by hand as a split application following each harvest as calcium ammonium nitrate (27 : 0 : 0 : 0; N : P : K : S). Applications were timed to coincide with either an irrigation or a rainfall event to wash the N fertiliser into the soil.

Plate 3.3 Pre (bottom) and post (top) mow with walker MC GHS lawnmower.

Table 3.6 Annual number of harvests, number of fertiliser applications and seasonal distribution based on forage type.

Forage type	Season	Dates	No. of Harvests	No. of fertiliser applications
Grasses and herbs	Summer	1 December - 28 February	3	3
	Autumn	1 March - 31 May	3	3
	Winter/early spring	1 June - 30 September	1	1
	Late spring	1 October - 31 November	2	2
Legumes	Summer	1 December - 28 February	2	2
	Autumn	1 March - 31 May	2	2
	Winter/early spring	1 June - 30 September	1	1
	Late spring	1 October - 31 November	2	2

Table 3.7 Single N fertiliser application rates to plots.

Grasses and herbs		Legumes	
Annual N application rates	**Single N application rates**	**Annual N application rates**	**Single N application rates**
(Kg N/ha/year)	(Kg N/ha/application)	(Kg N/ha/year)	(Kg N/ha/application)
0	0	0	0
45	5	39	6
90	10	78	11
180	20	156	22
315	35	272	39
450	50	389	56

3.2.3 Herbage measurements

At each harvest, one 3.00 m × 0.45 m strip was cut with a Briggs and Stratton 650 series 190cc rotary blade push mower to a height of 4 cm and herbage collected in the catcher in each plot (Plate 3.4). The fresh weight of herbage was recorded and two subsamples of 50 – 100 grams (g) of fresh weight were taken. The first subsample was weighed fresh and oven dried at 60 degrees centigrade (°C) for 48 hours (h) to determine DM percentage (DM %) (Adesogan *et al.* 2000). The DM % was multiplied by fresh weight to determine herbage DM yield. The herbage DM yield was calculated on a seasonal basis using the daily growth rates (kg DM/ha/day) of each harvest, multiplied by the total days in the season. The second subsample was frozen and freeze dried before being ground through a 1 mm sieve with a M200 rotor mill (Retsch Inc., Newtown, Pennsylvania, USA) and scanned by near-infrared spectrophotometry (NIRS, NIRSystems 5000, Foss, Maryland, USA) to determine herbage crude protein (CP) concentration. The NIRS calibration used to calculate the CP of the herbage was calculated from previous experiments not included in this thesis that used similar herbage samples. A different calibration was used for the herb samples than the legume and grass samples. When samples were outside the calibration spectrum, wet chemistry was performed by combustion under oxygen (Elemental Analyser vario MAX CN, Analysensysteme GmbH. Hanau, Germany). Nitrogen concentration of the herbage was calculated

by dividing CP by 6.25 and data for each plot was calculated on a seasonal basis using the average N concentration in each season.

Plate 3.4 Mowing the strip of a plot using the Briggs and Stratton 650 series 190cc rotary blade push mower to a height of 4 cm (a). Herbage was collected in the catcher and transferred to be weighed for herbage DM yield (b).

Total monthly rainfall and average monthly air temperature from 1 December 2014 to 30 November 2016 at LURDF, Lincoln, Canterbury, New Zealand was collected from Broadfields Meteorological Station, 1 kilometre (km) from research site.

3.2.4 Statisitcal analysis

The effects of N fertiliser rate, forage and their interactions on individual herbage DM were compared by multiple linear regression with groups (forage), (Genstat Version 16, VSN International Ltd) by using the following model:

$y = \alpha_j + \beta_j x_j + e_j,$

where y is independent variable (herbage DM yield), α is the overall mean, β is the block (n = 3), j is the plant forage (n = 12), x is N fertiliser rate (n = 6) and e is the residual error (n = 3).

The effects of N fertiliser rate, forage and their interactions on individual herbage N concentrations were compared by quadratic regression with groups (forage), (Genstat Version 16, VSN International Ltd) by using the following model:

$y = \alpha_j + \beta_j x_j + \beta_j x_j^2 + e_j,$

Where y is the independent variable (herbage N concentration), α is the overall mean, β is the block, j is the plant forage, x is N fertiliser rate and e is the residual error.

3.3 Results

3.3.1 Meteorological data

Total rainfall during year 1 (401 mm) was lower than the average long-term rainfall of the last 30 years (599 mm, Figure 3.1). Rainfall in year 2 (510 mm) was also lower than the average long-term rainfall (Figure 3.1 (a)). The monthly air temperature showed a similar trend to the long-term average air temperature (Figure 3.1 (b)). Both years were hotter compared to the 30-year long term average, particularly in year 2 which averaged 2 ^{o}C warmer from February 2016 to June 2016.

Lower rainfall in year 1 was made up with 105 mm more irrigation compared to year 2 (Table 3.8). Total water applied to the plots (rainfall and irrigation) was 1082 mm in year 1 and 1086 mm in year 2.

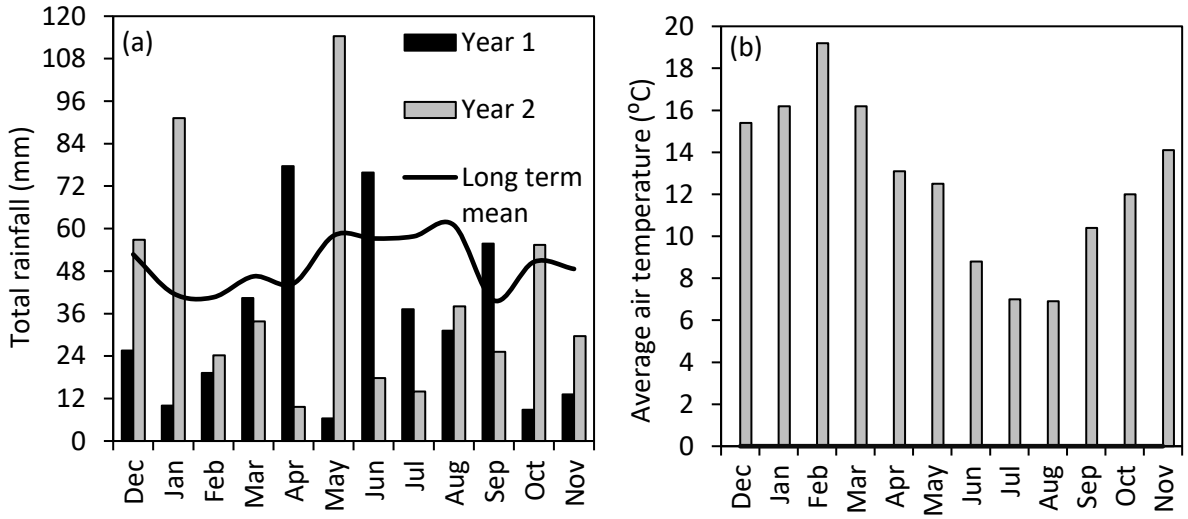

Figure 3.1 Total monthly rainfall (a) and average monthly air temperature (b) from year 1 (December 2014 - November 2015), year 2 (December 2015 - November 2016) and in comparison with long term mean (1981-2010) at LURDF, Lincoln, Canterbury, New Zealand. Data was collected from Broadfields Meteorological Station, 1 km from research site.

Table 3.8 Total monthly irrigation and water applied (rainfall + irrigation) to plots from year 1 (December 2014 to November 2015) and year 2 (December 2015 to November 2016) at LURDF, Lincoln, Canterbury, New Zealand. Data was collected from Broadfields Meteorological Station, 1 km from research site.

	Irrigation (mm)		Total water applied (mm)	
Month	**Year 1**	**Year 2**	**Year 1**	**Year 2**
December	110	80	135.6	136.8
January	107	82	117	173.2
February	105	100	124.2	124.2
March	70	109	110.4	142.8
April	35	20	112.6	29.6
May	10	10	16.4	79.6
June	10	0	85.8	61.2
July	0	0	37.2	12.2
August	0	0	31.2	41.2
September	20	10	75.8	35.2
October	94	63	102.8	118.4
November	120	102	133.2	131.6
Total	**681**	**576**	**1082**	**1086**

3.3.2 Herbage DM yield

The statistical effects of treatments on annual and seasonal herbage DM yield of the forages are presented in the Appendix (Table A. 1).

Averaged across all N fertiliser rates, annual herbage DM yield in year 1 was highest (P<0.001) in legumes and prairie grass (11801 - 13213 kg DM/ha/year), intermediate in herbs, cocksfoot and Italian ryegrass (9686 kg - 10520 kg DM/ha/year) and low in tall fescue, tetraploid perennial ryegrass, diploid perennial ryegrass and high sugar perennial ryegrass (8286 – 9131 kg DM/ha/year) (Figure 3.2). Annual herbage DM yield in year 2 was highest (P<0.001) in red clover and lucerne (9013 – 9802 kg DM/ha/year), intermediate in cocksfoot, plantain, prairie grass and white clover (87879 – 8646 kg DM/ha/year) and lowest in chicory, high sugar perennial ryegrass, tetraploid perennial ryegrass and diploid perennial ryegrass (7759 – 6905 kg DM/ha/year). However, when comparing maximum yield potential (450 or 389 kg N/ha/year) among all the forages, prairie grass, Italian ryegrass and plantain were the highest yielding forages (P<0.001) in year 1 (19213 – 15232 kg DM/ha/year) and prairie grass and Italian ryegrass were the highest yielding forages (P<0.001) in year 2 (13675 – 13116 kg DM/ha/year), producing more DM than the legumes (13149 kg DM/ha/year in year 1 and 8723 kg DM/ha/year in year 2).

Between the two years, annual herbage DM yield, was 2223 kg DM/ha/year higher (P<0.001) in year 1 than year 2 (Figure 3.2). Moreover, seasonal herbage DM yields in the forages differed between forages and N fertiliser rates. These are explained in more detail below.

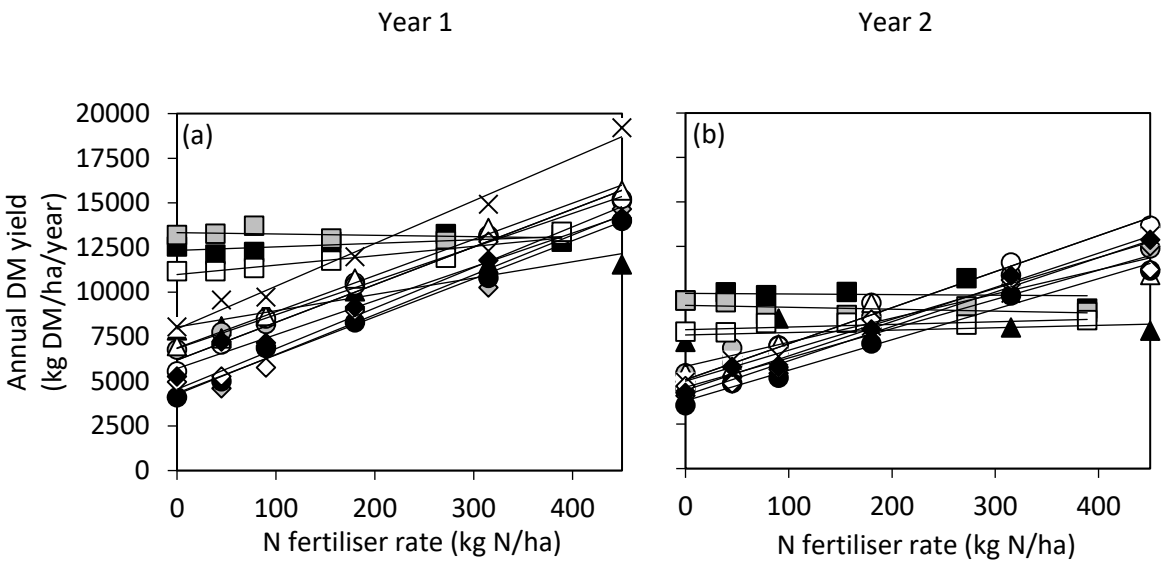

Figure 3.2 Linear effects of N fertiliser rate on annual herbage DM yield in year 1 (a) and year 2 (b) for the following 12 pasture forage: chicory (▲), plantain (Δ), lucerne (■), white clover (□), red clover (■), diploid perennial ryegrass (●), Italian ryegrass (○), cocksfoot (●), high sugar perennial ryegrass (◆), tall fescue (◆), tetraploid perennial ryegrass (◊) and prairie grass (X). Regression equation and R^2 for each forage are found in Appendix (Table A. 1)

There was a significant ($P < 0.001$) interaction between N fertiliser rate and forage for annual herbage DM yield. This was particularly noted at the lower N fertiliser rates (0 – 180 kg N/ha/yr) (Figure 3.2). In year 1, annual herbage DM yield of the lucerne and red clover was unaffected by N fertiliser rate. However, in the other 10 forages, annual herbage DM yield increased linearly with each fertiliser rate. More specifically chicory and white clover were similar but low in average DM responses to N (2.9 - 9.9 kg DM/kg N, averaged over the N fertiliser rates), plantain, tetraploid perennial ryegrass and cocksfoot, were intermediate in average DM responses to N (16.5 – 19.4 kg DM/kg N, averaged over the N fertiliser rates) and high sugar perennial ryegrass, diploid perennial ryegrass, prairie grass, tall fescue and Italian ryegrass were the highest in average DM responses to N (22.5 – 27.7 kg DM/kg N, averaged over the N fertiliser rates) (Table 3.9). In year 2, annual herbage DM yield of legumes and chicory were unaffected by N fertiliser rate. However, in the grasses and plantain, there was a significant linear relationship to increasing N fertiliser rates in herbage DM yield ($P<0.001$). More specifically tetraploid perennial ryegrass had a low DM response to N (13.5 kg DM/kg N, averaged over the N fertiliser rates), plantain, prairie grass, high sugar perennial ryegrass, cocksfoot and diploid perennial ryegrass had intermediate DM responses to N (15.2 – 20.1 kg DM/kg N, averaged over the N fertiliser rates) and tall fescue and Italian ryegrass had the highest DM responses to N (21.7 – 25.8 kg DM/kg N, averaged over the N fertiliser rates) (Table 3.9).

Table 3.9 DM response rates to N fertiliser rate of 12 forages at six different N fertiliser rates.

Year 1	**N rate - grasses and herbs**	**45**	**90**	**180**	**315**	**450**	**Average**	**Linear R^2**	**Quadratic R^2**
	N rate - legumes	**39**	**78**	**156**	**272**	**389**			
		----------kg DM/kg N applied----------							
	Chicory	5.1	12.3	12.2	11.8	8.2	9.9	0.01	0.68
	Plantain	9.4	18.8	21.0	21.0	19.4	17.9	0.34	0.78
	Cocksfoot	21.8	16.0	20.8	19.9	18.5	19.4	0.02	0.02
	HSG	10.6	31.8	27.3	19.4	23.4	22.5	0.01	0.14
	Italian RG	33.2	32.9	26.8	24.2	21.5	27.7	0.94	0.97
	Prairie grass	33.4	18.5	21.9	21.8	24.8	24.1	0.05	0.42
	Diploid PRG	20.0	30.6	23.3	21.2	21.9	23.4	0.1	0.11
	Tetraploid PRG	6.6	8.8	22.0	24.9	20.2	16.5	0.58	0.96
	Tall fescue	43.6	21.0	21.7	20.7	19.7	25.4	0.38	0.62
	Lucerne	-11.8	-4.1	1.7	2.6	0.7	-2.2	0.53	0.94
	Red clover	2.0	6.7	-1.2	-1.2	0.1	1.2	0.32	0.46
	White clover	0.0	2.3	3.7	2.8	5.7	2.9	0.73	0.74
	Average	14.5	16.3	16.8	15.8	15.3			
Year 2	**N rate - grasses and herbs**	**45**	**90**	**180**	**315**	**450**	**Average**	**Linear R^2**	**Quadratic R^2**
	N rate - legumes	**39**	**78**	**156**	**272**	**389**			
		----------kg DM/kg N applied----------							
	Chicory	-7.1	14.6	7.1	2.6	1.4	3.7	0.01	0.2
	Plantain	7.1	17.7	21.9	17.2	12.2	15.2	0.01	0.74
	Cocksfoot	31.5	17.3	17.7	17.5	15.6	19.9	0.42	0.61
	HSG	22.8	13.9	21.0	20.7	18.7	19.4	0	0.01
	Italian RG	32.0	26.9	27.0	22.7	20.5	25.8	0.89	0.91
	Prairie grass	26.9	16.2	17.5	15.9	18.3	19.0	0.22	0.6
	Diploid PRG	27.6	17.1	19.3	19.6	16.8	20.1	0.34	0.42
	Tetraploid PRG	3.2	10.3	20.8	18.9	14.5	13.5	0.33	0.93
	Tall fescue	32.5	16.0	19.8	20.9	19.1	21.7	0.17	0.33
	Lucerne	11.6	3.9	3.0	4.6	-1.2	4.4	0.61	0.63
	Red clover	-2.3	-9.9	-5.2	-1.2	-1.7	-4.1	0.29	0.31
	White clover	-1.1	6.2	3.2	1.5	1.7	2.3	0.01	0.13
	Average	15.4	12.5	14.4	13.4	11.3			

Diploid PRG, diploid perennial ryegrass; Tetraploid PRG, Tetraploid perennial ryegrass; HSG, high sugar perennial ryegrass; Italian RG, Italian ryegrass.

Averaged over the two years and all N fertiliser rates, seasonal herbage DM yield of all the forages was higher in summer compared to the other seasons, with values ranging from 2784 kg DM/ha (diploid perennial ryegrass) to 4877 kg DM/ha (white clover). There was a significant interaction ($P < 0.001$) between N fertiliser rate and forage for summer herbage DM yield. In year 1, whilst herbage DM yield showed an increased linear response to N fertiliser rate in grasses, herbs and white clover, summer herbage DM yield was unaffected by N fertiliser in lucerne and red clover (Figure 3.3 (a)). In year 2, there was a significant linear increase in herbage DM yield in grasses and plantain. However, in legumes and chicory there was no relationship (Figure 3.3 (b)).

In autumn, herbage DM yield ranged from 1253 kg DM/ha (diploid perennial ryegrass) to 1671 kg DM/ha (prairie grass), averaged over the two years and all N fertiliser rates. A significant interaction (*P* < 0.001) between N fertiliser rate and forage occurred in autumn herbage DM yield. In year 1, herbage DM yield of lucerne and red clover was unaffected by N fertiliser. However, in the other forages, herbage DM yield increased linearly with each N fertiliser rate in both years (Figure 3.3 (c)). In year 2, herbage DM yield was unaffected by N fertiliser in legumes and chicory. However, in grasses and plantain an increasing linear response occurred (Figure 3.3 (d)).

Herbage DM yield was lowest in winter/early spring ranging from 698 kg DM/ha (chicory) to 1450 kg DM/ha (Italian ryegrass), averaged over the two years and all N fertiliser rates. A significant interaction (P<0.001) between N fertiliser rate and forage occurred in winter/early spring herbage DM yield. Whilst legumes were not affected by N fertiliser in both years 1 and 2, chicory was also unaffected in year 2, and herbage DM yield of the remaining forages increased as N fertiliser rates increased (Figure 3.3 (e) and (f)).

In late spring herbage DM yield in the forages ranged from 2582 kg DM/ha (white clover) to 3116 kg DM/ha (lucerne), averaged over the two years and all N fertiliser rates. A significant interaction (P<0.001) between N fertiliser rate and forage occurred in late spring herbage DM yield. Legumes were unaffected by N fertiliser rate in both year 1 and 2, and chicory also unaffected in year 2. However, herbage DM yield of the grasses and plantain increased with increasing fertiliser rates in both years (Figure 3.3 (g) and (h)).

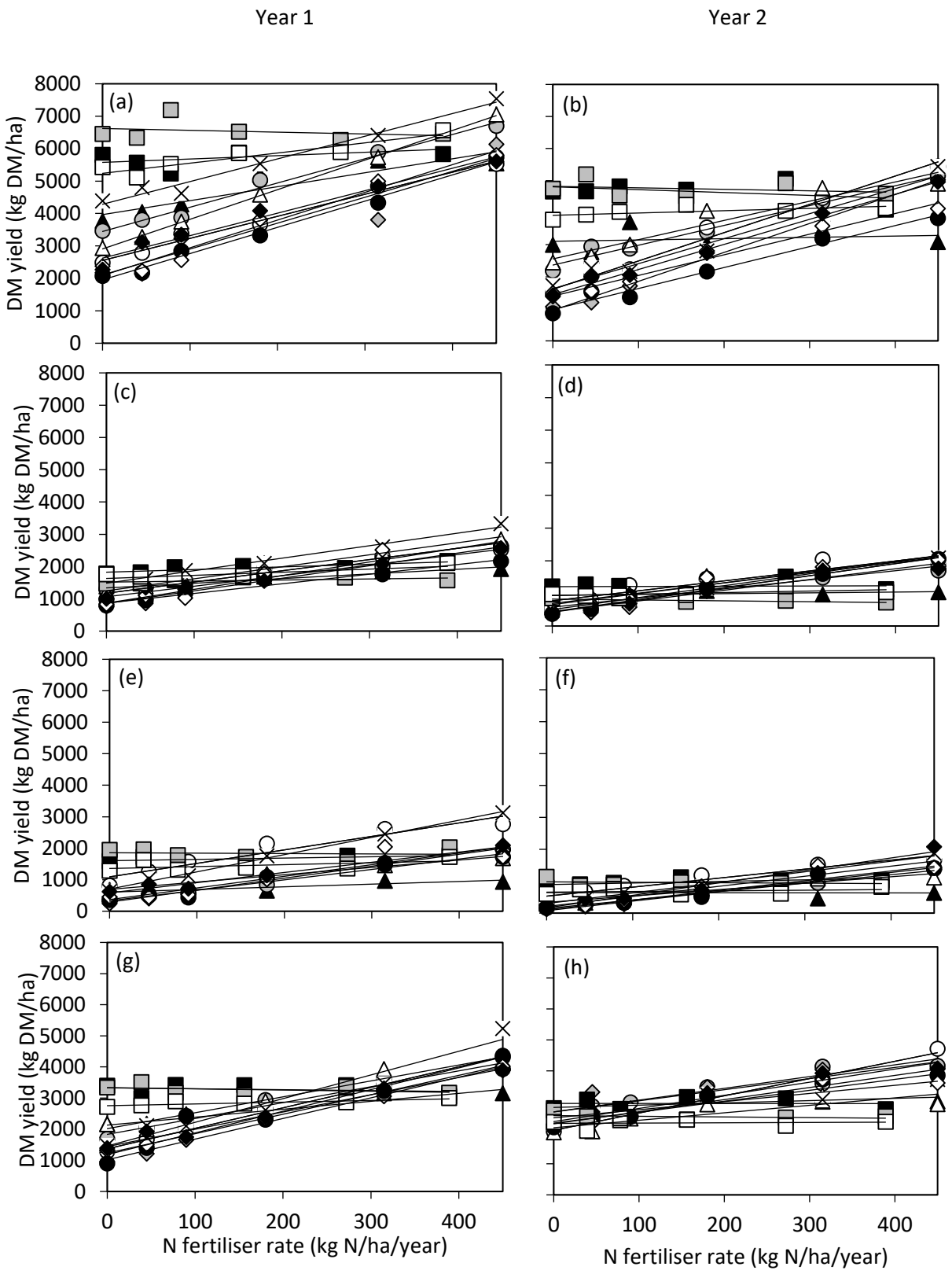

Figure 3.3 Linear effects of N fertiliser rate on herbage DM yield in summer (a and b), autumn (c and d), winter/early spring herbage (e and f) and late spring (g and h) for the following 12 pasture forage: chicory (▲), plantain (Δ), lucerne (■), white clover (□), red clover (■), diploid perennial ryegrass (●),Italian ryegrass (○), cocksfoot (●), high sugar perennial ryegrass (◆), tall fescue (◆), tetraploid perennial ryegrass (◊) and prairie grass (X). Regression equation and R^2 for each forage are found in the Appendix (Table A. 1).

3.3.3 Herbage N concentration

The statistical effects of treatments on annual and seasonal herbage N concentration of the forages are presented in the Appendix (Table A. 2).

Averaged over the two years and all N fertiliser rates, annual herbage N concentration was greatest in legumes (4.3 – 4.5 % N), intermediate in chicory and cocksfoot (3.1 - 3.5 % N), and lowest in plantain and other grasses (2.5 – 2.8 % N), (Figure 3.4). There was a significant interaction between N fertiliser rate and forage for annual N concentration of the herbage. The N concentration of the legumes was unaffected by N fertiliser. However, in herbs and grasses, there was a quadratic relationship to increasing N fertiliser rates in annual herbage N concentration. In both years, up to 180 kg N/ha/year N fertiliser rate, no difference occurred in annual average herbage N concentration of grasses and herbs. However, at N fertiliser rates above 180 kg N/ha/year for the grasses and herbs, annual herbage N concentration slowly increased. However, herbage N concentration of legume, averaged over the year, was unaffected by N fertiliser.

Average N concentrations, which differed between each season, are explained in more detail below.

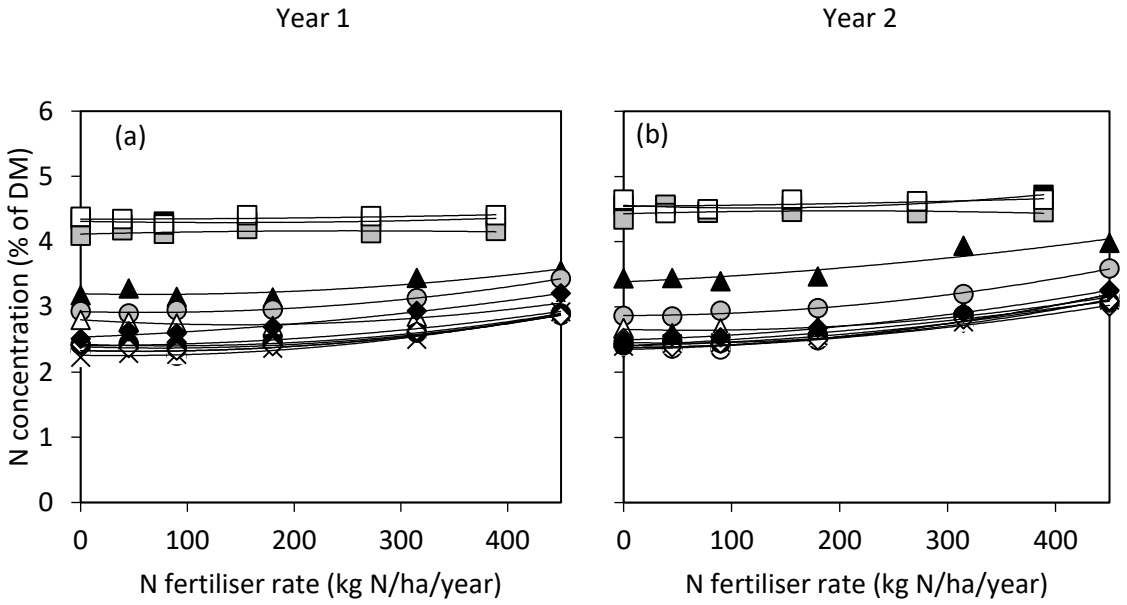

Figure 3.4 Quadratic effects of N fertiliser rate on annual N concentration of herbage averaged over year 1 (a) and year 2 (b) for the following 12 pasture forage: chicory (▲), plantain (Δ), lucerne (■), white clover (□), red clover (■), diploid perennial ryegrass (●),Italian ryegrass (○), cocksfoot (●),high sugar perennial ryegrass (◆), tall fescue (◆), tetraploid perennial ryegrass (◊) and prairie grass (X). Regression equation and R^2 for each forage are found in Appendix (Table A. 2).

Averaged over the two years and all N fertiliser rates, seasonal herbage N concentration in the summer ranged from 2.3 % N (diploid perennial ryegrass, tetraploid perennial ryegrass, high sugar perennial ryegrass and Italian ryegrass) to 4.2 % N (white clover). There was a significant interaction ($P<0.001$) between N fertiliser rate and forage for summer herbage N concentration. In year 1, an increasing

quadratic relationship between N fertiliser rate and N concentration were shown for cocksfoot, high sugar perennial ryegrass, plantain, prairie grass and tetraploid perennial ryegrass. Up to 180 kg N/ha/year N fertiliser rate, no difference occurred in annual average herbage N concentration. However, at N fertiliser rates above 180 kg N/ha/year, annual herbage N concentration slowly increased (Figure 3.5 (a)). However, N concentration in the legumes, chicory, Italian ryegrass, diploid perennial ryegrass and tall fescue did not response to N fertiliser. Conversely, in year 2 only plantain and tall fescue had quadratic responses to N fertiliser, increasing after N fertiliser rates of 180 kg N/ha/year were applied, and all other forages were not affected by N fertiliser (Figure 3.5 (b)).

Herbage N concentrations of all the forages except chicory was highest in autumn, with values ranging from 3.0 % N (Italian ryegrass) to 4.9 % N (white clover), averaged over the two years and all N fertiliser rates. There was a significant ($P<0.001$) interaction between N fertiliser rate and forage for autumn herbage N concentration. In year 1, herbage N concentration for Italian ryegrass, prairie grass and diploid perennial ryegrass had significant quadratic responses to N fertiliser rate increasing after 180 kg N/ha/year was applied. However, in legumes, herbs and the other grasses (tetraploid perennial ryegrass, tall fescue, high sugar perennial ryegrass and cocksfoot) did not respond to N fertiliser (Figure 5.3 (c)). In year 2, herbage N concentration in all forages, except legumes, diploid perennial ryegrass and tall fescue, had an increasing quadratic response to N fertiliser rate after the 180 kg N/ha/year treatment (Figure 3.5 (d)).

Herbage N concentration in the winter/early spring ranged from 2.5 % N (prairie grass and Italian ryegrass) to 4.9 % N (lucerne), averaged over the two years and all N fertiliser rates. There was a significant ($P<0.001$) interaction between N fertiliser rate and forage for winter/early spring N concentration of the herbage. In year 1, a quadratic response was shown for herbage N concentration in diploid perennial ryegrass which increased in N concentration after 180 kg N/ha/year. However, no quadratic N responses ($P>0.05$) were shown in the other forages and therefore N concentration was similar in all N fertiliser rates (Figure 3.5 (e)). In year 2, N concentration of Italian ryegrass, plantain, prairie grass, red clover and tetraploid perennial ryegrass produced an increasing quadratic response to N fertiliser rate, increasing after 180 kg N/ha/year. However, legumes, chicory, diploid perennial ryegrass, tall fescue high sugar perennial ryegrass and prairie grass did not respond to N fertiliser rate with similar N concentration at all the N rates (Figure 3.5 (f)).

Herbage N concentration of all the forages except red clover and lucerne was lowest in late spring with values ranging from 2.2 % N (prairie grass and high sugar perennial ryegrass) to 4.1 % N (lucerne and white clover), averaged over the two years and all N fertiliser rates. There was a significant interaction ($P<0.001$) between N fertiliser rate and forage for late spring N concentration of the herbage. In year 1, herbs, tetraploid perennial ryegrass, cocksfoot, tall fescue, prairie grass and Italian ryegrass had

quadratic responses between herbage N concentration and N fertiliser and therefore increased after 180 kg N/ha/year was applied. However, legumes, diploid perennial ryegrass and high sugar perennial ryegrass had no response to N and remained similar at all N fertiliser rates (Figure 3.5 (g)). In year 2, herbage N concentration in cocksfoot, Italian ryegrass, prairie grass, tetraploid perennial ryegrass and lucerne showed an increasing quadratic effect to N fertiliser, increasing after 180 kg N/ha/year was applied. However, both herbs, white clover, red clover, tall fescue, diploid perennial ryegrass and high sugar perennial ryegrass did not have quadratic responses to N fertiliser rates and remained similar in herbage N concentration throughout at all N fertiliser rates (Figure 3.5 (h)).

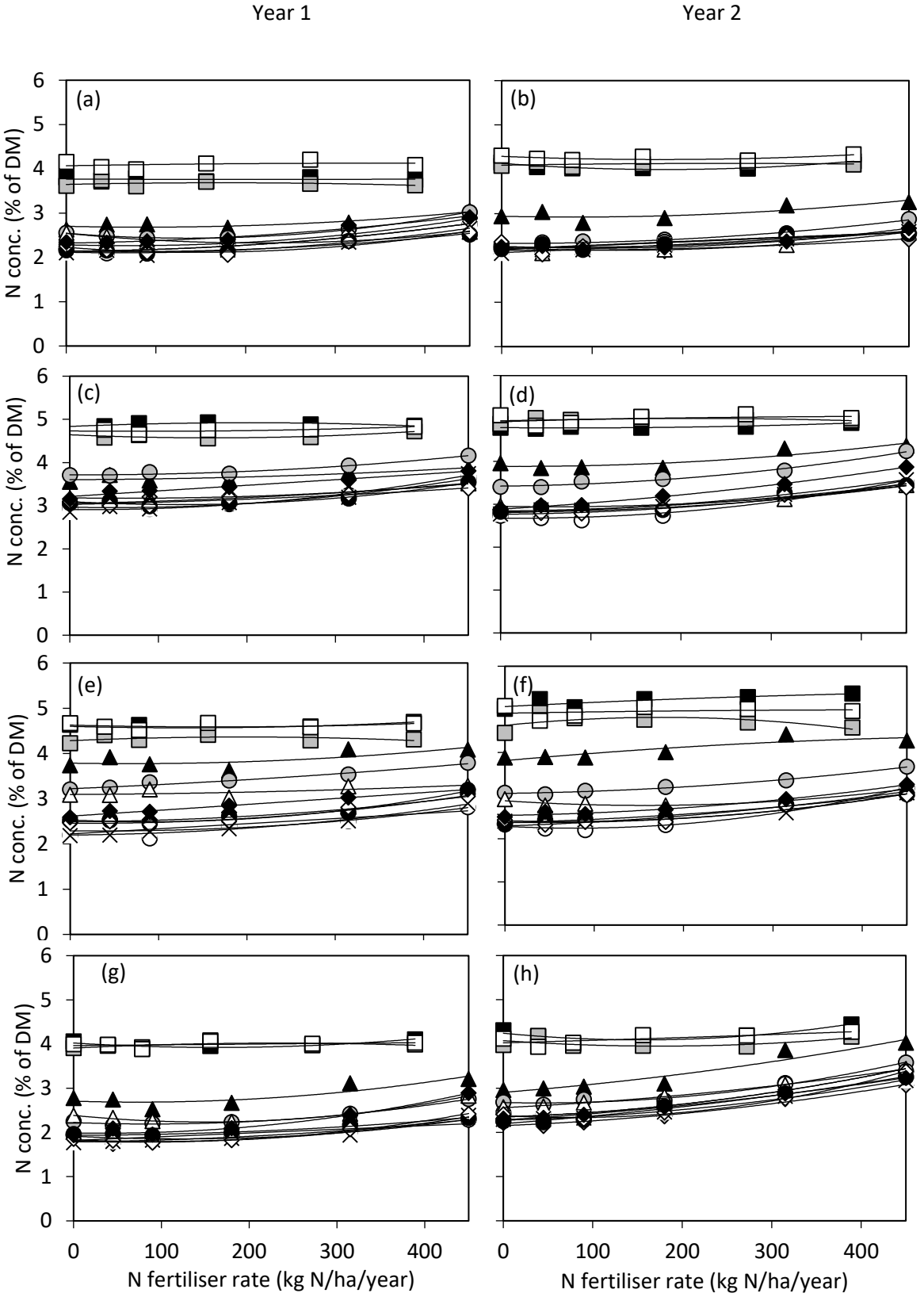

Figure 3.5 Quadratic effects of N fertiliser rate on herbage N concentration (conc.) in summer (a and b), autumn (c and d), winter/early spring (e and f) and late spring (g and h) for the following 12 pasture forage: chicory (▲), plantain (Δ), lucerne (■), white clover (□), red clover (■), diploid perennial ryegrass (●),Italian ryegrass (○), cocksfoot (●), high sugar perennial ryegrass (◆), tall fescue (◆), tetraploid perennial ryegrass (◊) and prairie grass (X). Regression equation and R^2 for each forage are found in Appendix (Table A. 2).

3.4 Discussion

3.4.1 Herbage DM yield

Higher growth rates in year 1 compared to year 2 in all the forages meant there was a 22 % decrease in herbage DM yield in the second year of the experiment. This was likely due to a combination of factors which include higher weed burden, a wet January in 2016 (year 2) and lower soil fertility. In year 2, there was a higher percentage of weeds observed, which therefore likely suppressed herbage DM yield of the sown forages. The plots were significantly lower in phosphorous and sulphur at the end of the experiment (see soil test results, section 3.2.2, Table 3.3) due to no super phosphate fertiliser applied in the second year. This may have restricted growth and herbage DM yield (Grant *et al.* 2001).

Poor persistence of herbage DM yield was especially prevalent in prairie grass which grew 34 % less herbage DM yield in year 2 than year 1. One potential reason for this is grazing frequency. Fulkerson *et al.* (2000) and Turner *et al.* (2006) suggested prairie grass needs a longer regrowth intervals of 49-56 days or 3.5–4.0 leaves/tiller grazing interval (time to emergence of one new leaf was 14 days) to allow for adequate seed setting in the summer and optimal plant survival. However, in the current study, grazing intervals were shorter than this (25 – 35 days) which may have affected the persistence of the prairie grass. In addition, the invasion of grasses weeds such as *Poa annua* which could not be chemically removed from the prairie grass pure swards may have also affected its persistence. This result which was also shown in Fulkerson *et al.* (2000).

Poor persistence was also evident in chicory, with a yield decrease from 9686 kg DM/ha/yr in year 1 to 7759 kg DM/ha/yr in year 2, which may have been due to weed invasion. Chicory is especially susceptible to competition from weeds and these are difficult to remove using chemicals because of limited selection of herbicide.

Forage effect

Across all N fertiliser rates and over the two years, a higher herbage DM yield occurred in legumes than in herbs and grasses, with legumes producing an average of 2050 kg DM/ha/year more herbage DM annually than grasses, and 1714 kg DM/ha/year more annual herbage than herbs. In regards to individual forages, total annual herbage DM yields differed in order of lucerne > red clover > prairie grass > white clover > cocksfoot > plantain > Italian ryegrass > chicory > tall fescue > tetraploid perennial ryegrass > high sugar perennial ryegrass > diploid perennial ryegrass. High summer growth rates of 58 kg DM/ha/day (averaged over the 2 years) were the key reason behind high legume herbage DM yields, which were consistent with previous research (Orr *et al.* 1990). The herbage DM yield potential of lucerne over perennial ryegrass and herbs has been well documented under both irrigated

and dryland conditions, especially under low soil N conditions (Brown *et al.* 2005; Hayes *et al.* 2010; Mills and Moot 2010). However, what has often been overlooked is the high herbage DM yield of white clover monocultures compared with perennial ryegrass monocultures. Brock (1973) showed that white clover yield was 9760 – 12 010 kg DM/ha/year under dryland conditions. This illustrated the ability of legumes to fix their own N as well as growing rapidly in the summer, when rainfall is adequate.

The high herbage DM yields in prairie grass and Italian ryegrass were due to high growth rates in autumn and winter/early spring (Fraser 1982; Malcolm *et al.* 2014; Vartha 1977; Woods *et al.* 2016) and reflects their cool season growth ability (Plate 3.5). The implication of this is that these two forages have the potential to decrease N losses in the soil in the autumn and winter/early spring by taking up more N from the soil at a time of year when traditional perennial ryegrass - white clover pastures are not growing. This was shown in Malcolm *et al.* (2014) where nitrate leaching losses beneath Italian ryegrass were 24 – 54 % less than other pasture species (perennial ryegrass – white clover, tall fescue – white clover and diverse pasture). This was attributed to greater N uptake rates through greater winter activity in Italian ryegrass compared to the other species in the experiment.

Plate 3.5: Differences in herbage DM yield of Italian ryegrass (left) and diploid perennial ryegrass (right) in early spring

Higher growth rates of plantain in autumn is contributed to a higher annual herbage DM yield compared to perennial ryegrass and also suggests that plantain is more tolerant to the cooler autumn

temperatures (Plate 3.6). The high herbage DM yield of plantain in autumn has been shown previously (Goh and Bruce 2005; Minneé *et al.* 2013) and may be specific to the cultivar (Tonic) used in this study. The cultivar Tonic has been bred to be more winter active (Stewart 1996). In addition, high herbage DM yields in plantain could also be explained by the chemical aucubin which has been found previously in plant leaves of plantain (Navarrete *et al.* 2016; Stewart 1996; Tamura and Nishibe 2002). Aucubin was discovered to cause an N inhibitory effect on soil N mineralisation (Dietz *et al.* 2013) leading to a higher concentration of ammonium present in the soil, increasing plant N uptake and herbage DM yield. This suggests that plantain has the capability to increase plant N uptake and decrease N losses in the soil during autumn, when other species are unable to because of cooler temperatures and lower growth rates.

Plate 3.6: Differences in herbage DM yield of plantain (left), diploid perennial ryegrass (centre) and prairie grass (right) in early spring.

A 7 % lower annual herbage DM yield in chicory compared to plantain is likely to be due to winter dormancy in chicory (Lancashire and Brock 1983). This is shown in Lee *et al.* (2015) where, over an 18 month period, herbage DM yields were 23.0 t DM/ha for chicory and 29.8 t DM/ha for plantain. Likewise, a study by Labreveux *et al.* (2004) in Pennsylvania showed plantain had a higher annual herbage DM yield than chicory (9470 kg DM/ha vs 9023 kg DM/ha). Although selected for winter activity (Rumball *et al.* 2003), the cultivar Choice (sown in the current experiment) is known to produce poor winter herbage DM yields as shown in Lancashire and Brock (1983). This was shown in the current experiment with low winter growth rates of 5.8 kg DM/ha/day in the winter/early spring.

N fertiliser effect

Contrasting effects on N fertiliser were found on herbage DM yield, with grasses and herbs showing an increasing response, while legumes were unresponsive. This resulted in grasses and herbs yielding more than legumes at the high N fertiliser rates. Averaged over the two years herbage DM yield ranged from 11,481 – 13,812 kg DM/ha/year at 450 kg N for grasses and herbs but was 10,936 kg DM/ha/year at 389 kg N/ha/year for legumes.

The average N response in grasses and herbs across all N rates and over the two years was 6.8, 16.6, 15.0, 19.9, 21.0, 23.5, 21.7, 21.5 and 26.8 kg DM/kg N for chicory, plantain, tetraploid perennial ryegrass, cocksfoot, high sugar perennial ryegrass, tall fescue, diploid perennial ryegrass, prairie grass and Italian ryegrass, respectively.

The average N response to DM of chicory was lower than reported previously (10.6 to 13.2 kg DM/kg N applied) (Clark *et al.* 1990; Collins and McCoy 1997). The reason for low DM response to N in chicory is unclear but may reflect the higher herbage DM yield of chicory at a low N input in the current experiment, than what has been recorded earlier. With no N fertiliser, annual herbage DM yield of chicory was 7501 kg DM/ha/year and greater than the grasses (+ 2485 kg DM/ha) and plantain (+ 1313 kg DM/ha). Further, experiments in mixed pastures at the same site showed a lower response to N fertiliser in diverse pastures (0.9 kg DM/kg N applied), which contained less than 20 % chicory, compared to perennial ryegrass–white clover pasture (8.0 kg DM/kg N applied) (Van Rossum *et al.* 2013). Alternatively, the lower N response in chicory could have reflected cutting interval. Previous N response experiments have used cutting intervals of 5–6 weeks compared with 4 weeks in the current experiment.

The lack of an effect of N fertiliser on legumes has been noted previously and reflects the ability of legumes to fix N for their N requirements (McKenzie *et al.* 1999). Legumes may use N from N fertiliser when available rather than from biological N fixation (Schwinning and Parsons 1996), which means that total N in the plant maybe unaltered. However, it is important to note that biological N fixation in legumes will not completely cease when N fertiliser is adequate for high herbage DM yields (Armstrong *et al.* 1999). An increase in yield from applied fertiliser N has been found in irrigated and unirrigated lucerne and white clover swards (Cowling 1961; Hoglund *et al.* 1974). Previous experiments have shown that an N response was due to lack of rhizobia in the soil (Hoglund *et al.* 1974), cold temperatures which supresses biological N fixation in early spring (Hoglund *et al.* 1974; Williams 1932; Young 1958) or soils being low in organic matter (Hannaway and Shuler 1993).

3.4.2 Herbage N concentration

Forage effect

The herbage N concentration, averaged over the two years and all fertiliser rates, was greatest in legumes (4.4 % N), intermediate in chicory and cocksfoot (3.1 – 3.5 % N) and lowest in grasses and plantain (2.5 – 2.8 % N). The higher N concentration of legumes versus grasses and herbs at all N rates is consistent with the results of previous experiments (Elgersma and Hassink 1997; Fraser and Rowarth 1996). While this high N concentration in legumes is important for pasture growth, it does contribute to a high herbage N intake by cows grazing mixed pastures, with consequent high rates of N excretion in urine (Kebreab *et al.* 2001). Previous short term experiments by Totty *et al.* (2013) and Woodward *et al.* (2012) have shown that feeding diverse pastures containing herbs (chicory, plantain) in addition to perennial ryegrass–white clover, can result in lower urinary N excretion from dairy cows in late lactation than feeding primarily perennial ryegrass–white clover. This was proposed to be because herbs have a lower N concentration, thus reducing the herbage N intake of a diverse pasture (Beukes *et al.* 2014). However, in the current experiment, conducted under irrigation, herbage N concentrations in chicory and plantain were higher or equal to grasses, including perennial ryegrass. In turn, this may lead to higher herbage N intakes per unit of DM consumed in herbs than grasses. This indicates that factors other than herbage N intake may drive lower N excretion in livestock grazing pasture containing herbs (Box *et al.* 2016; Stewart 1996; Tamura and Nishibe 2002). These include secondary compounds such as catapol which has been suggested to effectively act as a diuretic causing animals to urinate more frequently, thus lowering the N concentration of the urine (Deaker *et al.* 1994). In addition, the bioactive compound acteoside has been suggested to improve rumen fermentation and use N as an energy source rather than it being converted into urea and lost in the urine (Navarrete *et al.* 2016). A higher water concentration in plantain and chicory has also suggested to be the reason for more frequent urination events causing less concentrated N in the urine (Cheng *et al.* 2017; O'connell *et al.* 2016). This result was also shown in the current experiment where, averaged throughout the year, DM % of the herbage was 7 % lower in plantain and chicory compared to grasses (Table A.3).

Averaged over the two years and all N fertiliser rates, herbage N concentration for all the forages in the autumn was above the recommended herbage N intake for cows in late lactation to reduce N losses in the urine (3 % N) (Castillo *et al.* 2001). This was due to slower growth rates from cooler temperatures causing lower rates of N dilution and lower rates of N mobilisation (Hoekstra *et al.* 2007; Peyraud and Astigarraga 1998). However, in the winter/early spring period N concentration in the grasses was below 3 % N. This was not expected due to cool temperatures in winter (June – August) restricted grass growth. However, the winter/early spring period did include a September harvest which was 2.3^{o}C warmer in temperature and therefore triggered higher herbage DM yields. This likely caused higher N

dilution rates and lower N concentration in the plant. As a result, feeding animals grass forages instead of legumes or herbs in the winter/early spring period may have less of an impact on urinary N excretion.

N fertiliser effect

There were contrasting effects of N fertiliser on the herbage N concentration for the different species; legumes were unresponsive to N fertiliser, whereas the herbage N concentration of herbs and grasses increased after 180 kg N/ha/year was applied. This may have depleted soil N, so that although herbage DM yield increased with low rates of N application, an increase of N concentration did not occur until relatively high rates of N were applied. This also suggests herbage N concentrations are difficult to alter and that using N fertiliser as a mitigation strategy to decrease herbage N concentration may be an ineffective way to reduce herbage N intake (Shepherd and Lucci 2013).

The average herbage N concentration was higher in cocksfoot and chicory (2.9 – 3.8 % N) than in grasses and plantain (2.4 – 3.1 % N), which is consistent with the results of previous experiments (Belesky *et al.* 2000; Collins and McCoy 1997; Sanderson *et al.* 2003). This may reflect an improved N uptake by these plants with deeper and more vigorous rooting systems. Alternatively, it may be a consequence of the harvesting regime, which was similar for grasses and herbs. The unresponsiveness of N concentration in legumes to N fertiliser was likely due to their ability to fix N and therefore contain optimum amounts in the herbage for growth. This was demonstrated by Clement *et al.* (2016) in an experiment at the same site, which noted the herbage N concentration of perennial ryegrass -white clover fertilised with 156 kg N/ha/year was similar to perennial ryegrass -white clover pasture fertilised with 304 kg N/ha/year (3.5 % N vs 3.6 % N).

3.5 Conclusions and implications

The main implications drawn from this experiment are:

- At all N fertiliser rates, the N concentration was highest in legumes, intermediate in herbs and lowest in grasses.

- Legumes had a higher herbage DM yield than grasses and herbs at fertiliser rates up to 272 kg N/ha/year but did not respond to N fertiliser.

- The addition of N fertiliser increased the herbage DM yield of grasses and herbs however, herbage N concentrations were more difficult to alter suggesting a decrease N fertiliser usage may be an ineffective way to reduce herbage N intake.

- The results suggested that for the irrigated Canterbury environment, legumes in pastures could be a contributor to high herbage N intake by animals and that there were no large benefits from using herbs instead of grasses in reducing herbage N intake of livestock when DM intakes are the same.

Chapter 4

Effects of regrowth interval and nitrogen fertiliser on dry matter yield and chemical composition of alternative pasture forages

4.1 Introduction

Reducing nitrate leaching from agricultural land is an important goal for New Zealand farmers to ensure they are within environmental regulations developed by Regional Councils across New Zealand (Ministry for the Environment 2014). These regulations will require large reductions in nitrogen (N) inputs and outputs which may affect profitability. Nitrogen fertilisers are often used in farm systems to maintain annual herbage dry matter (DM) yield. However, their excessive use is discouraged due to the impact on N loss. In Chapter 3, the effect of N fertiliser on herbage DM yield and N concentration was compared in a suite of forages at optimum time of defoliation and found interactions between species and N fertiliser rate. The results suggested different species may benefit from different management strategies to achieve animal production targets under a regulated N loss regime.

Nitrate leaching in pasture-based dairy systems occurs largely from urine patches due to large quantities of mineral N deposited (Di and Cameron 2002b). The risk of leaching is greatest in the autumn when cooler temperatures reduce plant N uptake and higher rainfall increases drainage of soil mineral N. Plant characteristics, such as high annual herbage DM yield and winter activity, have been identified to improve capture of soil N (Malcolm *et al.* 2014; Woods *et al.* 2016) as well as low herbage N concentration to reduce urine N (Woods *et al.* 2016). Both annual herbage DM yield and plant characteristics can be altered using farm management practices. These management practices include timing of grazing, otherwise known as regrowth interval, which causes a change in plant chemical composition and growth habits. In perennial ryegrass, the changes in chemical composition include a decrease in herbage N concentration (Ball *et al.* 2012; Bryant *et al.* 2012; Wilman 1975), an increase in water soluble carbohydrate (WSC) concentrations (Fulkerson and Slack 1994) and a decrease in digestibility (Fulkerson and Donaghy 2001) as regrowth interval increases. In addition, Bryant *et al.* (2012) also showed interactions between regrowth stage and N fertiliser on herbage N concentration in perennial ryegrass. However, the experiment found that the herbage quality declined during regrowth when tested with dairy cows, and lead to a reduced milk yield (Bryant *et al.* 2014). While regrowth interval and N fertiliser rate experiments are well documented in perennial ryegrass, less information exists on alternative pasture forages, species such as plantain, chicory, clover and other grasses to reduce N losses without impeding quality.

Therefore, the objective of this experiment was to quantify the effect of N fertiliser rate on herbage DM yield and chemical composition in plantain, chicory, red clover, white clover, diploid perennial ryegrass and cocksfoot at different regrowth intervals in spring and autumn.

4.2 Materials and methods

4.2.1 Experimental site and design

This experiment was conducted within the larger experiment described in Chapter 3 (section 3.2.1) using the same plots 12 months (10 March - 7 April 2015) and 18 months (13 October – 10 November 2015) after sowing. Of the 12 forages sown, six were used in this experiment. In brief, the six pasture forages in 2.1 m x 3.0 m plots were grown at three N fertiliser rates and harvested each week for 4 weeks using a split-split-plot factorial design with three replicates. The forages were selected for this experiment because they were either high in herbage DM yield (Plantain, white clover, red clover), low in N concentration (diploid perennial ryegrass), or responded differently than expected in Chapter 3. For example, cocksfoot and chicory were higher in N concentration than expected. The experiment was conducted under irrigation on a free-draining Templeton fine sandy loam (Immature Pallic soil) (Hewitt 2010) at LURDF, Canterbury, New Zealand (43°64'S, 172°46'E). Forages (Table 4.1) were the main plot treatments, N fertiliser treatments were the split-plot treatments and regrowth intervals were the split-split plot treatments. The N fertiliser rates were split into three categories. These were nil, medium and high N fertiliser. The nil N fertiliser rate was 0 kg N/ha/year for grasses, herbs and legumes. The medium N fertiliser rate was 180 kg N/ha/year for grasses and herbs and 156 kg N/ha/year for legumes. The high N fertiliser rate was 450 kg N/ha/year for grasses and herbs and 389 kg N/ha/year for legumes.

Table 4.1 Forages sown and their functional group, scientific name, cultivar and sowing rate.

Pasture	Functional group	Scientific name	Cultivar	Sowing rate (kg/ha)
Diploid PRG – late season flowering	Grass	*Lolium perenne*	One-50 (AR37)	20
Cocksfoot	Grass	*Dactylis glomerata*	Savvy	8
Chicory	Herb	*Cichorium intybus*	Choice	8
Plantain	Herb	*Plantago lanceolata*	Tonic	10
White clover	Legume	*Trifolium repens*	Kopu 2	5
Red clover	Legume	*Trifolium pratense*	Sensation	10

Diploid PRG, diploid perennial ryegrass.

Details of establishment and management are described in Chapter 3 (section 3.2.1). Briefly, the experimental area was sown in March 2014 following cultivation. Control of herbage mass commenced in spring 2014 using only mower harvests, thus avoiding trampling and nutrient recycling by livestock. Between the two regrowth experiments, harvest intervals in spring, summer and autumn for grasses and herbs were 32, 26 and 30 days, respectively, and for legumes were 41, 35 and 41 days, respectively, to allow sufficient regrowth (Donaghy and Fulkerson 1998; Lee *et al.* 2015; Moot *et al.* 2003). Nitrogen fertiliser was applied by hand following each harvest as calcium ammonium nitrate (27: 0 : 0 : 0; N : P : K : S), with the total annual N application rate split throughout the year. This is shown in Table

4.2. Climate data was collected from Broadfields Meteorological Station, 1 km from the experimental area. Annual irrigation was 550 mm, applied between October and March.

Table 4.2 Single N fertiliser rates to plots for three N fertiliser treatments in spring and autumn 2015.

	Annual N application		Single N application	
N fertiliser treatment	**Grasses and herbs**	**Legumes**	**Grasses and herbs**	**Legumes**
	--------- kg N/ha/yr ---------		------ kg N/ha/application -------	
Nil	0	0	0	0
Medium	180	156	20	22
High	450	389	50	56

4.2.2 Herbage measurements

Regrowth data in autumn was collected weekly between 17 March and 7 April 2015 and in spring collected weekly between 13 October and 3 November 2015 for all the forages. Herbage DM yield, botanical composition and plant characteristics were determined above cutting height by harvesting three quadrats (32 cm x 60 cm, Plate 4.1) per plot using electric hand shears, with an attachment set to 4 cm height to mimic grazing of cows (Plate 4.2). Harvesting occurred between 10:00 am and 12:00 pm avoiding previously harvested areas. Timing of harvest was done within two hours at the same time of day, to avoid inconstancy's associated with diurnal changes in the herbage chemical composition later in the day. Harvested herbage was kept in the shade, until all plots had been harvested, then transported to the laboratory for further processing. Two quadrats were subsampled for botanical composition (Plate 4.3) and then oven-dried at 60 °C for 48 h and weighed to determine herbage yield. The third quadrat was mixed, and a subsample was frozen and freeze-dried for chemical analysis. It was then ground through a 1 mm sieve with a M200 rotor mill (Retsch Inc., Newtown, Pennsylvania, USA) and scanned by near-infrared spectrophotometry (NIRS, NIRSystems 5000, Foss, Maryland, USA) to determine herbage crude protein (CP) concentration, WSC and digestible organic matter in the DM (DOMD), based on calibrations derived on the experimental herbages in Chapter 3 (section 3.3.3). Any samples outside the calibration spectrum, were analysed by wet chemistry using the methods described in Chapter 3 (section 3.2.3). Nitrogen concentration was calculated by dividing CP by 6.25.

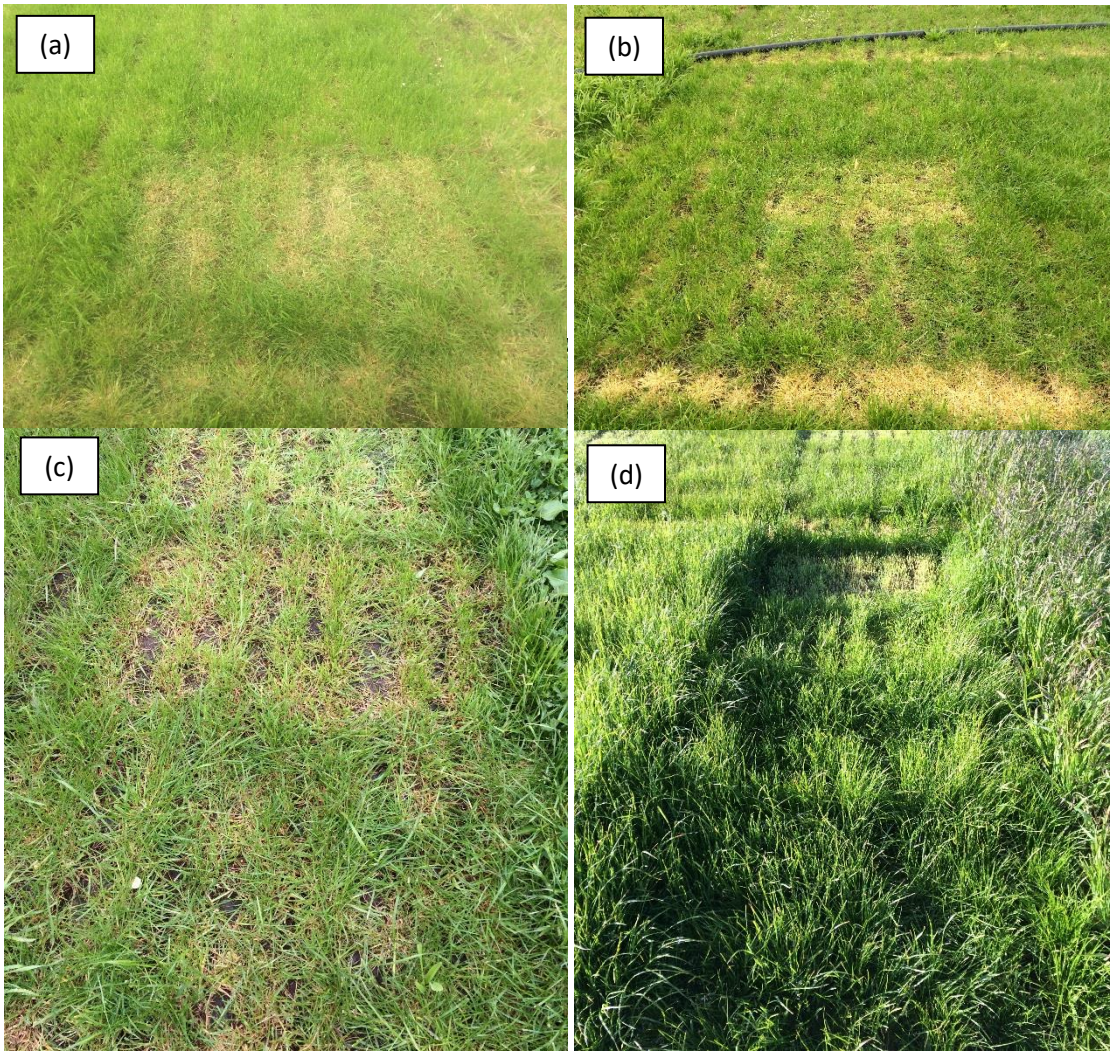

Plate 4.1 Perennial ryegrass quadrat cuts after 1 (a), 2 (b), 3 (c) and 4 (d) weeks of regrowth in spring.

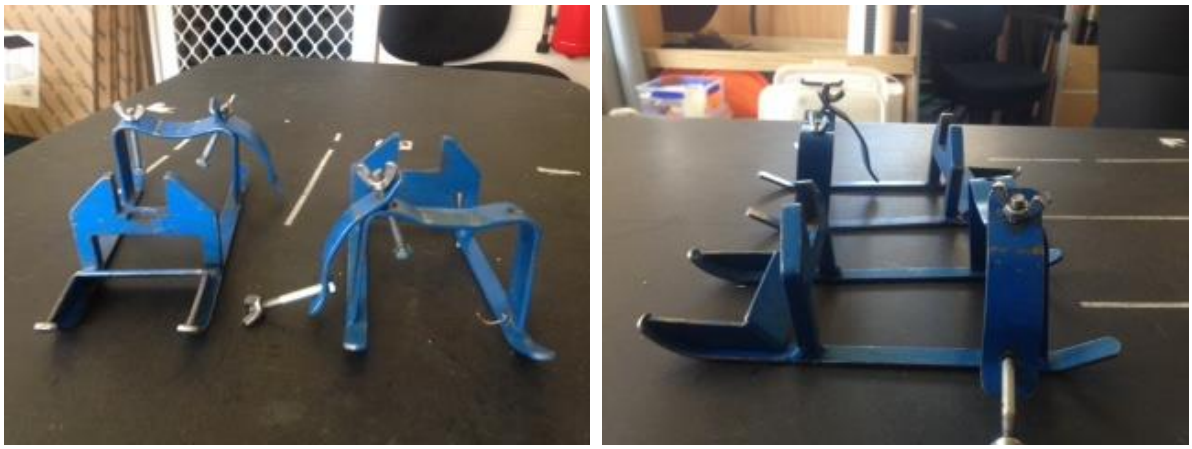

Plate 4.2 Attachment for electric hand shears set to 4 cm.

Plate 4.3 Botanical composition of white clover plot separated into sown species, grass weeds, broad leaf weeds and dead material.

4.2.3 Statistical analysis

The effects of N fertiliser treatment, forage and regrowth and their interactions on herbage DM yield, DM response to N, N concentration, WSC:CP ratio and DOMD were compared by ANOVA (Genstat Version 16, VSN International Ltd) using a split – split - plot model. In the statistical model, forage was the main plot factor (n=6), N fertiliser rate was the split plot factor (n=3) and regrowth interval was the split-split plot factor (n=4). Block was the residual error (n=3).

4.3 Results

4.3.1 Meteorological data

During the autumn experiment, temperatures were warmer and rainfall lower than the long-term mean (Table 4.3). However, during spring, temperature conditions were typical of an average year, though rainfall was low. More detailed conditions can be seen in Chapter 3 (section 3.3.1).

Table 4.3 Average daily air temperature, maximum and minimum air temperature, soil temperature and total rainfall from 10 March - 7 April 2015 (autumn experiment 2015) and 13 October - 14 November 2015 (spring experiment 2015) at LURDF, Lincoln, Canterbury, New Zealand. Long term averages were taken between 1981 – 2010 and averaged over two months. Data was collected from Broadfields Meteorological Station, 1 km from research site.

	Autumn		Spring	
	2015	**Long term mean**	**2015**	**Long term mean**
Average air temp (°C)	15.0	13.6	11.9	12.4
Max temp (°C)	19.9	18.8	17.3	17.6
Min temp (°C)	10.7	8.4	7.0	7.3
Average soil temp* (°C)	15.5	12.2	14.2	11.7
Total rainfall (mm)	20.8	45.5	19.0	49.6

* Soil temperature at 10 cm depth. Temp; temperature.

4.3.2 Botanical composition

At the final harvest (week 4) in autumn there was a significant difference ($P<0.1$) between forages showed the percentage of leaf was lower compared to the other forages. However, there were no other differences in botanical composition between the forages or N fertiliser rates.

Table 4.4 Botanical compositon of six forages at three N fertiliser treatments (nil, medium and high) in autumn at final regowth (week 4).

Forage	N rate		% Leaf.	% Stem.	% Dead.	% Weeds
Chicory	Low		84%	0%	2%	14%
Chicory	Medium		94%	0%	2%	5%
Chicory	HIgh		82%	0%	1%	18%
Cocksfoot	Low		90%	0%	4%	7%
Cocksfoot	Medium		86%	0%	4%	10%
Cocksfoot	HIgh		96%	0%	2%	2%
Plantain	Low		97%	0%	2%	1%
Plantain	Medium		98%	0%	2%	0%
Plantain	HIgh		99%	0%	1%	0%
Red clover	Low		61%	0%	2%	37%
Red clover	Medium		72%	3%	3%	22%
Red clover	HIgh		76%	0%	3%	22%
Diploid PRG	Low		93%	0%	6%	2%
Diploid PRG	Medium		93%	0%	5%	1%
Diploid PRG	HIgh		92%	0%	7%	0%
White clover	Low		84%	0%	1%	15%
White clover	Medium		95%	0%	3%	1%
White clover	HIgh		88%	0%	0%	12%
Autumn	Forage	F pr.	**	NS	NS	0.017
		LSD	**0.124**	0.015	0.031	0.139
	N rate	F pr.	NS	NS	NS	NS
		LSD	0.105	0.010	0.014	0.109
	Forage x N rate	F pr.	NS	NS	NS	NS
		LSD	0.236	0.023	0.040	0.249

NS, not significant; d.f, degrees of freedom; F pr., significance value.
* P≤0.05
** P<0.01
***P<0.001

In spring however, a significant interaction (P<0.01) between forage and N rate in stem showed cocksfoot, plantain and PRG decreased in the percentage of stem as N rate increased. Conversely, red clover increased as N fertiliser increased (Table 4.5).

In addition, differences in botanical composition between the forages found the percentage of leaf was significantly (P<0.01) lower in red clover compared with all other forages. This is likely due to large amounts of white clover that couldn't be removed by herbicide. Also, the percentage of stem was significantly (P<0.01) higher in plantain compared to the other forages. Finally, the percentage of dead matter was significantly lower in white clover compared to the other forages.

Differences in botanical composition between the N fertiliser rates also occurred. The percentage of leaf was significantly (P<0.01) lower in the low N fertiliser treatment compared to the medium and high N fertiliser treatments. Also, the percentage of stem was significantly (P<0.01) lower in the low N fertiliser treatment compared to medium and high N fertiliser treatments. Finally, the percentage of weeds was significantly (P<0.01) lower in the medium N fertiliser treatment compared to the low and high N treatments.

Table 4.5 Botanical compositon of six forages at three N fertiliser treatments (nil, medium and high) in spring at final regowth (week 4).

Forage		N rate	% Leaf.	% Stem.	% Dead.	% Weeds
Chicory		0	54%	0%	1.3%	44%
Chicory		200	83%	0%	1.0%	16%
Chicory		500	77%	0%	3.0%	20%
Cocksfoot		0	80%	17%	1.7%	1%
Cocksfoot		200	92%	4%	3.2%	1%
Cocksfoot		500	91%	3%	4.2%	2%
Plantain		0	64%	30%	0.7%	5%
Plantain		200	68%	26%	0.3%	5%
Plantain		500	81%	15%	0.8%	3%
Diploid PRG		0	81%	5%	2.9%	11%
Diploid PRG		200	94%	3%	2.1%	2%
Diploid PRG		500	93%	2%	2.4%	3%
Red clover		0	57%	3%	0.8%	39%
Red clover		200	64%	4%	2.2%	20%
Red clover		500	56%	6%	0.5%	38%
White clover		0	86%	0%	0.0%	14%
White clover		200	100%	0%	0.0%	0%
White clover		500	97%	0%	0.0%	3%
Forage	F pr.		*	**	**	NS
	LSD		**0.242**	**0.058**	**0.016**	0.238
N rate	F pr.		**	**	NS	**
	LSD		**0.075**	**0.028**	0.009	**0.073**
Forage x N rate	F pr.		NS	**	NS	NS
	LSD		0.273	**0.076**	0.023	0.268

NS, not significant; d.f, degrees of freedom; F pr., significance value.
* P≤0.05
** P<0.01
***P<0.001

4.3.3 Herbage DM yield

The statistical effects of treatments on herbage DM yield of the forages are presented in the Appendix Table B. 1 and Table B. 2.

Overall, there was an increasing effect of both regrowth (P<0.001) and N fertiliser (P<0.001) on herbage DM yield for all forages, (Figure 4.1 and Figure 4.2). In spring, a three-way interaction occurred between forage, N fertiliser and regrowth interval (P=0.011. Figure 4.2). The interaction was caused by low herbage DM yield of diploid perennial ryegrass and high herbage DM yield of legumes, at the low and medium N fertiliser rate (Figure 4.2 (a) and (b)). However, at the high N fertiliser rate the reverse was true with diploid perennial ryegrass exceeding herbage DM yield of legumes (Figure 4.2 (c)). Conversely in autumn, forages all responded the same way to regrowth interval at the three N fertiliser treatments meaning there was no three-way interaction (Figure 4.1 and Figure 4.2).

In both autumn and spring, responses between N treatment and regrowth interval (P<0.001) showed herbage DM yield was less effected by N fertiliser treatment earlier in the regrowth interval compared to the final harvest, where there were large differences between the N fertiliser treatments (Figure 4.1 and Figure 4.2).

Effects of regrowth interval between forages showed that, averaged over the N fertiliser rates and seasons, plantain and chicory grew more rapidly than forages such as diploid PRG and cocksfoot, therefore achieving higher herbage DM yields of 1544 kg DM/ha and 1517 kg DM/ha at 4 weeks compared to the lower yielding species (1146 and 1251 kg DM/ha) (P=0.03 in spring and P=0.013 in autumn, Figure 4.1 and Figure 4.2).

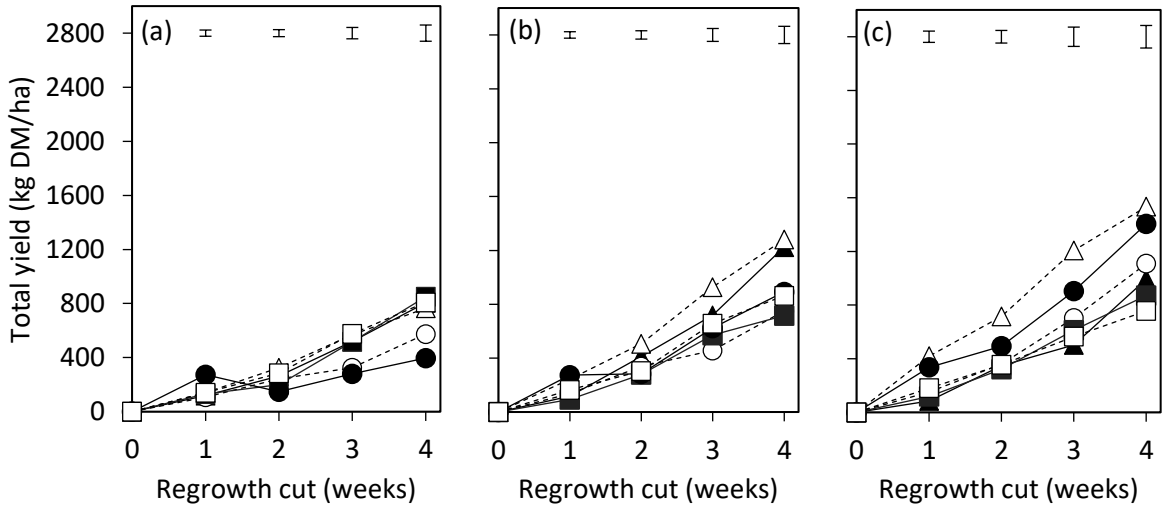

Figure 4.1 Effect of regrowth interval in autumn on total herbage DM accumulation at three N fertiliser treatments; nil (a), medium (b) and high (c), for six forages: diploid perennial ryegrass (—●—), cocksfoot (- -○- -), chicory (—▲—), plantain (- -Δ- -), red clover (—■—) and white clover (- -

□- -). Points are mean values. Error bars are standard error of the mean (SEM) of regrowth interval and fertiliser treatment, across all forages.

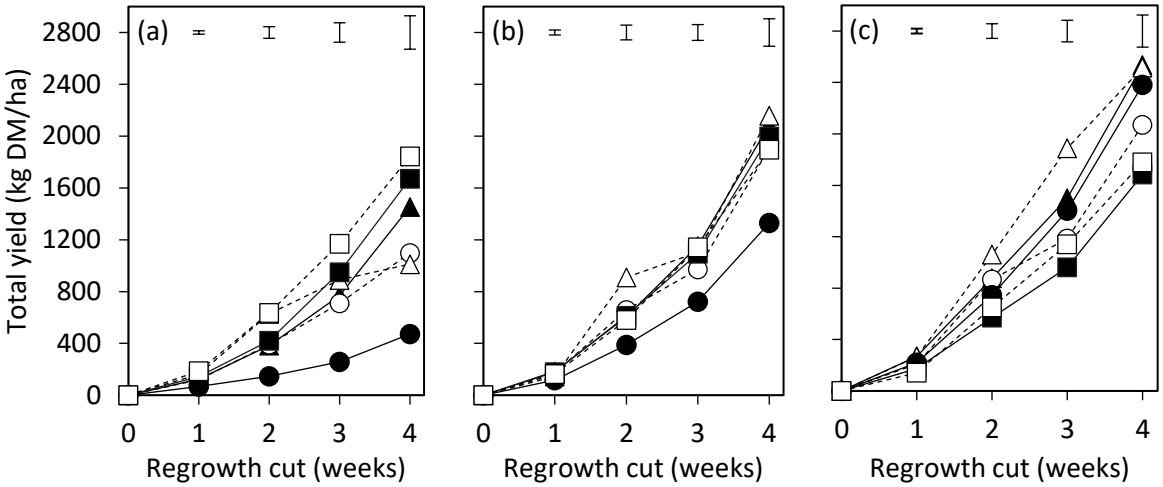

Figure 4.2 Effect of regrowth interval in spring on herbage DM accumulation at three N fertiliser treatments; nil (a), medium (b) and high (c), for six forages: diploid perennial ryegrass (—●—), cocksfoot (- -○- -), chicory (—▲—), plantain (- -Δ- -), red clover (—■—) and white clover (- -□- -). Points are mean values. Error bars are SEM of regrowth interval and fertiliser treatment, across all forages.

4.3.4 DM response rates to N

The statistical effects of treatments on DM response rates to N at 4 weeks of the forages are presented in Table 4.6.

Average DM responses to N were highest in plantain (20.6 kg DM/kg N in autumn and 43.7 kg DM/kg N in spring) and diploid perennial ryegrass (22.5 kg DM/kg N in autumn and 40.5 kg DM/kg N in spring) and lowest in legumes (-1.0 kg DM/kg N in autumn and 1.6 kg DM/kg N in spring, averaged over the two forages) ($P<0.001$).

In spring, there was a decrease in DM response rate when additional N fertiliser was applied ($P<0.001$) in all the forages (29.9 kg DM/kg N vs 18.1 kg DM/kg N), however the decrease was much smaller in legumes (3.6 kg DM/kg N vs -0.45 kg DM/kg N) compared to grasses (41.6 kg DM/kg N vs 28.8 kg DM/kg N) and herbs (44.5 kg DM/kg N vs 28.9 kg DM/kg N).

In autumn however, an interaction ($P<0.001$) between N fertiliser and forage for DM response revealed whilst there was no decrease in DM response rates between N fertiliser treatment for diploid perennial ryegrass, cocksfoot, plantain, white clover, red clover, there was a significant difference in chicory (21 kg DM/kg N vs 3.7 kg DM/kg N).

Table 4.6 DM response to N fertiliser in autumn and spring at two N fertiliser rate (medium and high N) and six forages.

	Autumn			Spring		
	N fertiliser treatment					
Forage	**Med**	**High**	**Average**	**Med**	**High**	**Average**
	----------kg DM/kg N applied----------					
Diploid PRG	24.8	20.2	22.5	42.8	38.2	42.8
Cocksfoot	8.8	10.7	9.8	40.4	19.4	40.4
Chicory	21	3.7	12.4	31.6	21.6	31.6
Plantain	25.8	15.4	20.6	57.3	30.1	57.3
White clover	2.5	-0.9	0.8	2.4	-1.1	2.4
Red clover	-6.1	0.4	-2.9	4.8	0.2	4.8
Average	12.8	8.3		29.9	18.1	
	P value	**LSD**		**P value**	**LSD**	
Forage	***	10.41		***	24.44	
N rate	**	3.63		***	6.98	
Forage x N rate	**	11.6		NS	26.2	

4.3.5 N concentration

The statistical effects of treatments on N concentration of the forages are presented in the Appendix Table B. 1 and Table B. 2.

Overall, herbage N concentration for the forages decreased as regrowth interval increased (P<0.001) and increased as N fertiliser increased (P<0.001) (Figure 4.3 and Figure 4.4). An interaction (P<0.001) between regrowth interval and N fertiliser in spring showed at the start of the regrowth interval, herbage N concentration was lowest in the low N treatment and was significantly different between all three N fertiliser treatments. However, by weeks 3 and 4 there was no difference in herbage N concentration in the nil and medium N treatments, indicating herbage N concentration decreased at a more rapid rate in medium N treatment compared to the other N rates (Figure 4.3 and Figure 4.4).

In autumn and spring, an interaction (P<0.001) between N fertiliser and forage revealed there was an increasing relationship between N fertiliser treatment and herbage N concentration for herbs and grasses with N being highest in chicory (4.0 % N) and lowest in diploid perennial ryegrass (3.3 % N) at the highest N treatment. However, there was no relationship for legumes which were always high in herbage N concentration (4.8 % N, Figure 4.3 and Figure 4.4).

Similarly, there was an interaction (P<0.001) between forage and regrowth on herbage N concentration. In autumn, herbage N did not change with regrowth interval in legumes. However, for both herbs and grasses the relationship between N concentration and regrowth showed in the first 2 weeks herbage N increased, then dropped by the fourth week (Figure 4.3, P=0.006). In spring, legumes did respond to regrowth interval decreasing over time, although not to the extent of grasses and herbs

which, in diploid perennial ryegrass and cocksfoot increased in the first two weeks, and then decreased, and in plantain and chicory a decreased from week 1 (Figure 4.4, P<0.001).

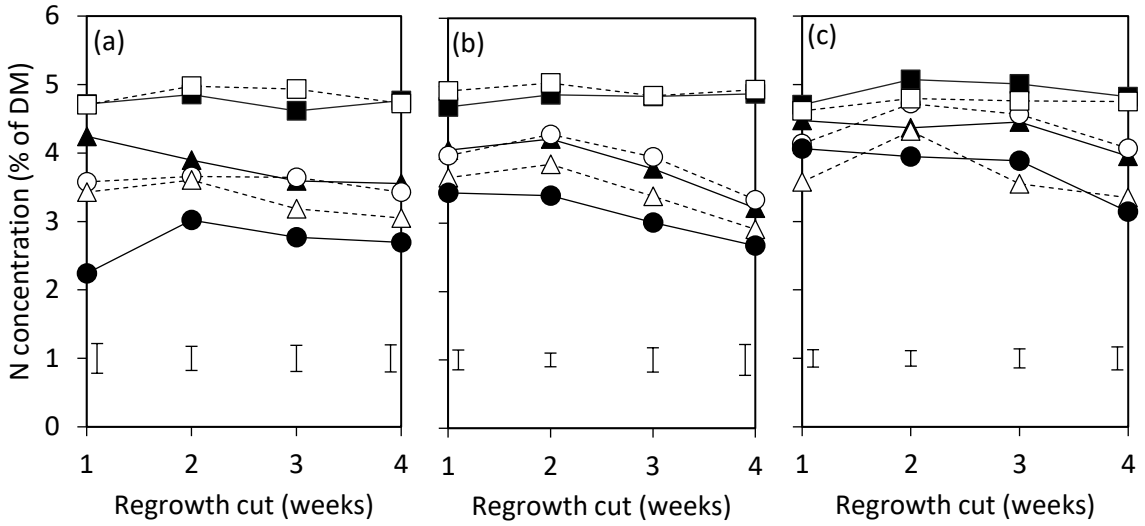

Figure 4.3 Effect of regrowth interval in autumn on N concentration at three N fertiliser treatments; nil (a), medium (b) and high (c), for six forages: diploid perennial ryegrass (—●—), cocksfoot (- -○- -), chicory (—▲—), plantain (- -Δ- -), red clover (—■—) and white clover (- -□- -). Points are mean values. Error bars are SEM of regrowth interval and fertiliser treatment, across all forages.

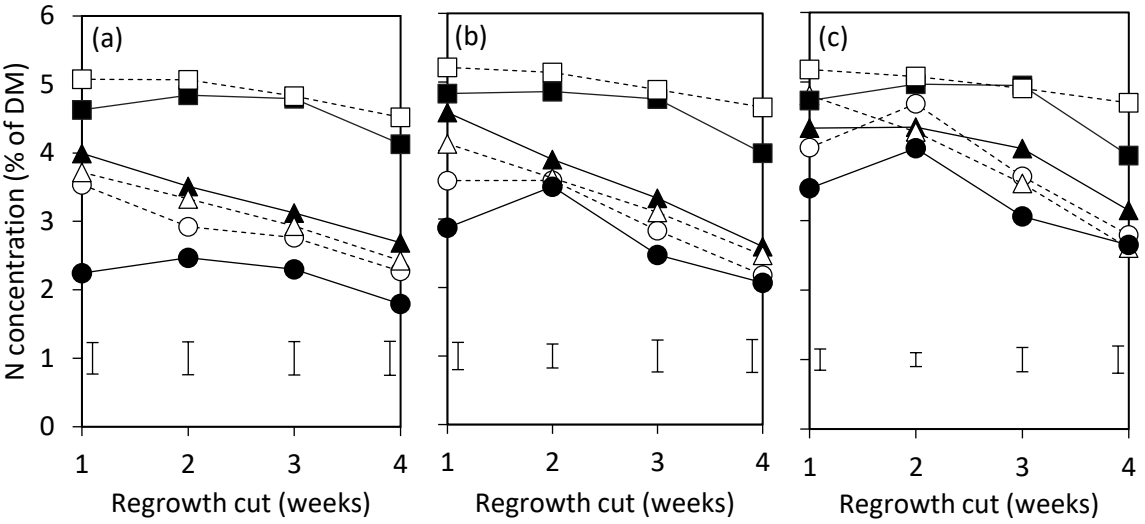

Figure 4.4 Effect of regrowth interval in spring on N concentration at three N fertiliser treatments; nil (a), medium (b) and high (c), for six forages: diploid perennial ryegrass (—●—), cocksfoot (- -○- -), chicory (—▲—), plantain (- -Δ- -), red clover (—■—) and white clover (- -□- -). Points are mean values. Error bars are SEM of regrowth interval and fertiliser treatment, across all forages.

4.3.6 WSC:CP ratio

As a measure of the balance between readily available energy (WSC) and N supply (CP) in animal diets, the ratio of WSC to CP was compared (Figure 4.5 and Figure 4.6). The statistical effects of treatments on the WSC:CP ratio of the forages are presented in the Appendix Table B. 1 and Table B. 2.

The effect of regrowth interval was different among forages ($P<0.001$) in both autumn and spring. In autumn, whilst an increasing effect of regrowth on WSCP: CP was shown in grasses, herbs and white clover, red clover was not affected by regrowth interval remaining at 0.3 from week 1 to 4. In addition, the effect of regrowth interval in cocksfoot and white clover WSC:CP ratios were considerably lower (0.3 to 0.4 from week 1 to 4) compared to the herbs and diploid perennial ryegrass (0.2 vs 0.9 from week 1 to 4). In spring, all forages increased in WSC:CP ratios as regrowth interval increased, however larger differences between some forages were found than others. For example legumes only increased in the WSC:CP ratio by 0.4 from week 1 to 4 (0.3 to 0.7 from week 1 to 4) whereas herbs and grasses increased by 1.1 (0.4 to 1.5 from week 1 to 4) with the highest increase from diploid perennial ryegrass (1.4) (Figure 4.5 and Figure 4.6).

An interaction between forage and N fertiliser treatment ($P=0.031$), showed that, in autumn, diploid perennial ryegrass decreased in WSC:CP ratio as N fertiliser increased (from 0.7 to 0.5), whereas the other forages were unaffected by N fertiliser remaining between 0.4 and 0.5 from nil to high N fertiliser rates. (Figure 4.5). However, in spring ($P<0.001$), both grasses decreased in WSC:CP ratio as N fertiliser increased (from 1.3 to 0.7), while herbs and legumes were unaffected (Figure 4.6).

The effect of N fertiliser and regrowth interval was significant in autumn ($P=0.037$), showing that while the WSC:CP ratio was highest in the nil N fertiliser treatment at the start of the regrowth interval (0.4), by the final harvest the medium N treatment was highest in WSC:CP ratio (0.7). However, this interaction did not occur in spring ($P>0.05$).

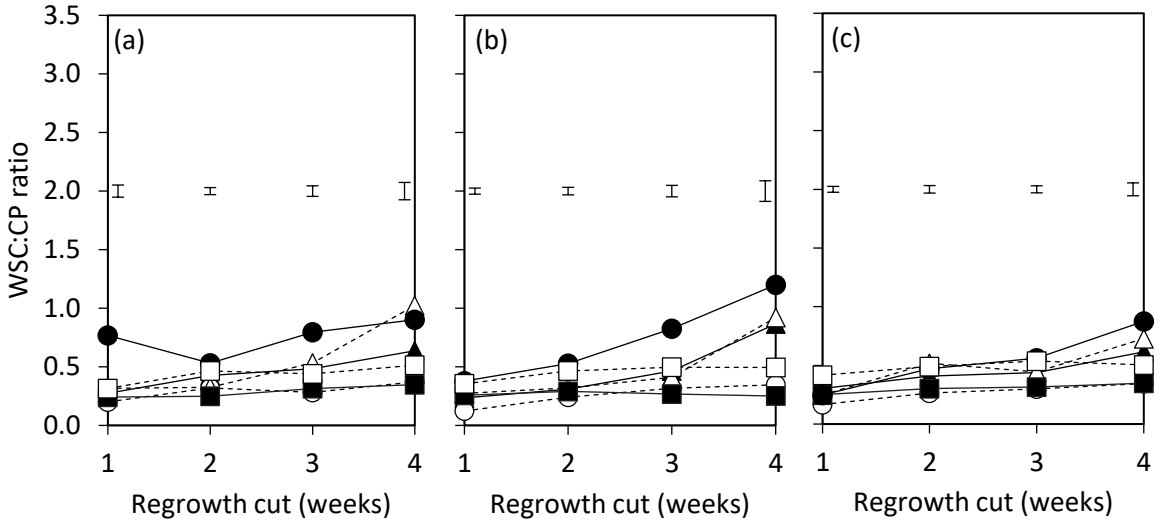

Figure 4.5 Effect of regrowth interval in autumn on the WSC:CP ratio at three N fertiliser treatments; nil (a), medium (b) and high (c), for six forages: diploid perennial ryegrass (—●—), cocksfoot (- -○- -), chicory (—▲—), plantain (- -Δ- -), red clover (—■—) and white clover (- -□- -). Points are mean values. Error bars are SEM of regrowth interval and fertiliser treatment, across all forages.

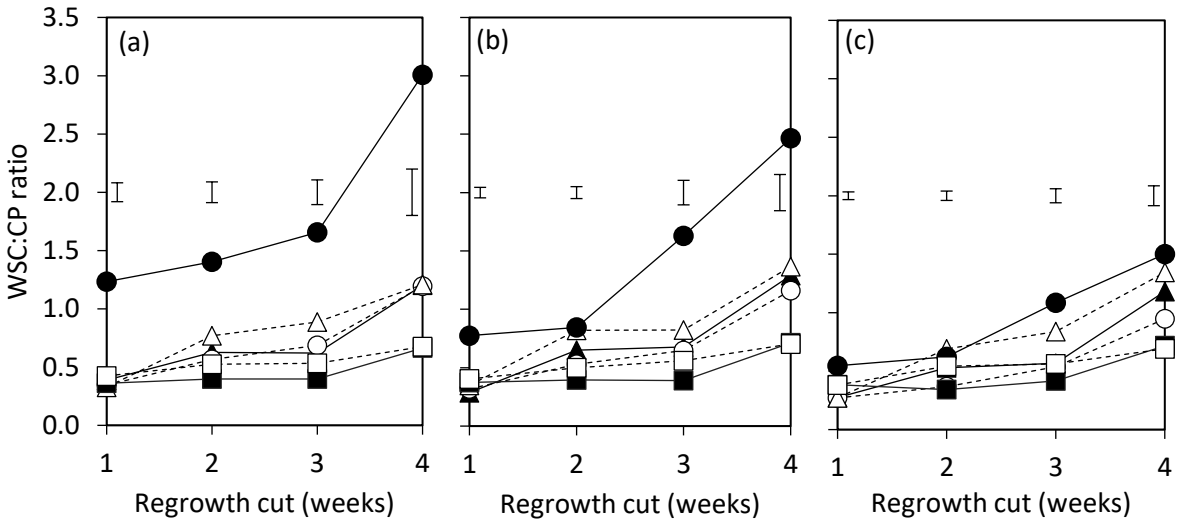

Figure 4.6 Effect of regrowth interval in spring on the WSC:CP ratio at three N fertiliser treatments; nil (a), medium (b) and high (c), for six forages: diploid perennial ryegrass (—●—), cocksfoot (- -○- -), chicory (—▲—), plantain (- -Δ- -), red clover (—■—) and white clover (- -□- -). Points are mean values. Error bars are SEM of regrowth interval and fertiliser treatment, across all forages.

4.3.7 **DOMD**

The statistical effects of treatments on DOMD of the forages are presented in the Appendix Table B. 1 and Table B. 2. Generally, DOMD was highest (P<0.001) in white clover (82.6 % in spring and 80.2 % In autumn) and lowest in cocksfoot (71.1 % in spring and 70.4 % in autumn) (Table B. 2, Figure 4.7 and Figure 4.8).

An interaction among forages at different N fertiliser treatments (P=0.023) occurred in spring. While no response to N fertiliser occurred in chicory, diploid perennial ryegrass, white clover and red clover,

DOMD in cocksfoot and plantain had an increased response to N fertiliser at the higher N fertiliser rates (Figure 4.7 and Figure 4.8).

An interaction between regrowth interval and forage (P<0.001) showed in autumn, there was no change in DOMD for red clover while for the other forages, DOMD increased from week 1 to week 4. Plantain produced the largest response to regrowth interval whereas cocksfoot and white clover showed the least response. In spring, the interaction between regrowth interval and forage (P<0.001) showed legumes were unresponsive to regrowth interval. However, there was an increase in DOMD in herbs and grasses from week 1 to week 4 (Figure 4.7 and Figure 4.8).

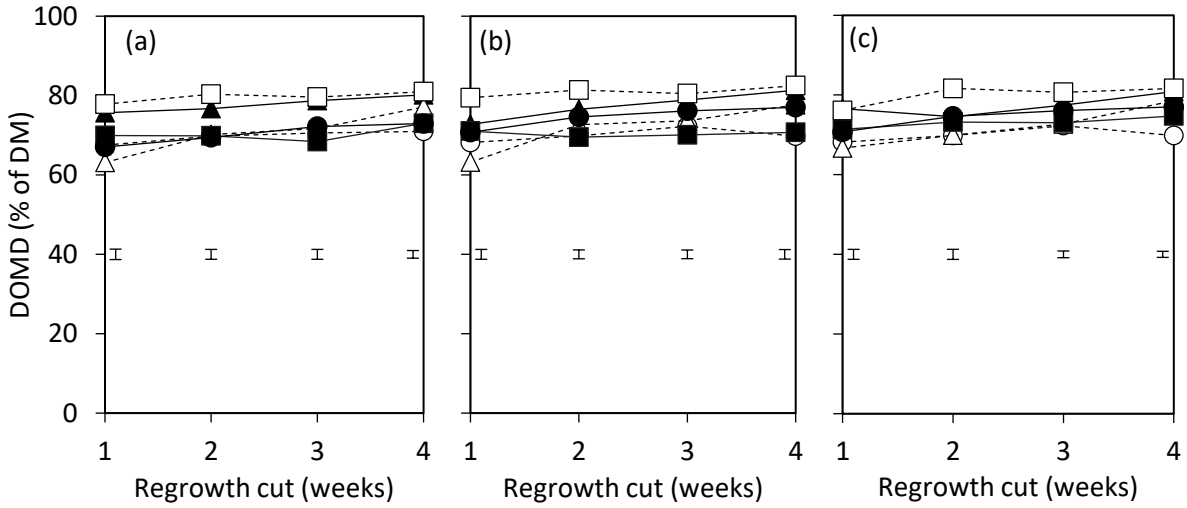

Figure 4.7 Effect of regrowth interval in autumn on DOMD at three N fertiliser treatments; nil (a), medium (b) and high (c), for six forages: diploid perennial ryegrass (—●—), cocksfoot (- -○- -), chicory (—▲—), plantain (- -Δ- -), red clover (—■—) and white clover (- -□- -). Points are mean values. Error bars are SEM of regrowth interval and fertiliser treatment, across all forages.

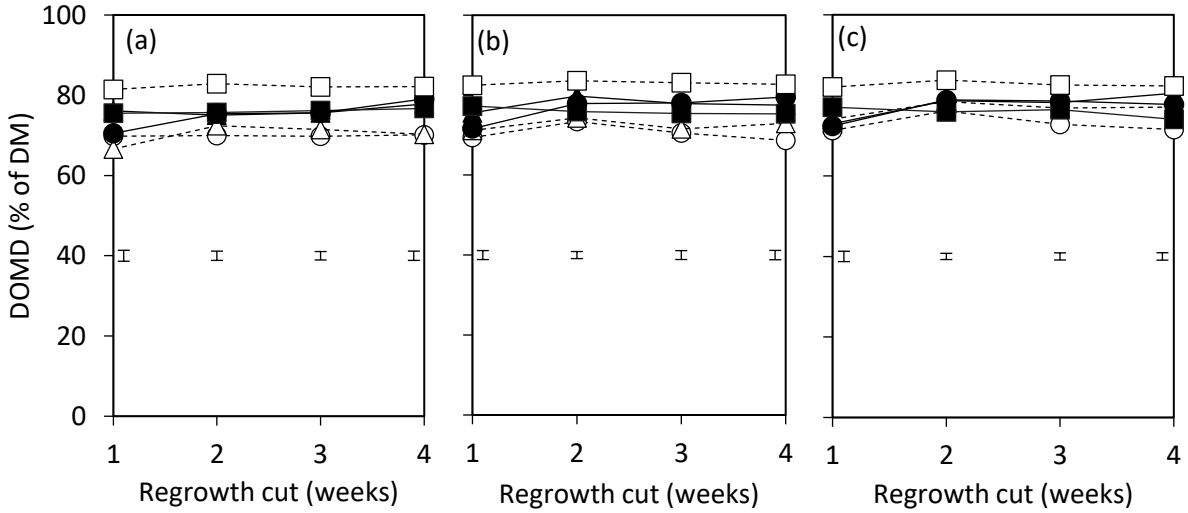

Figure 4.8 Effect of regrowth interval in spring on DOMD at three N fertiliser treatments; nil (a), medium (b) and high (c), for six forages: diploid perennial ryegrass (—●—), cocksfoot (- -○- -), chicory (—▲—), plantain (- -Δ- -), red clover (—■—) and white clover (- -□- -). Points are mean values. Error bars are SEM of regrowth interval and fertiliser treatment, across all forages.

4.4 Discussion

4.4.1 Herbage DM yield

Forage effect

Under nil N fertiliser, herbage DM yield was greatest for the legumes, white clover and red clover. This was expected and indicated N fertiliser does not limit herbage DM yield in legumes (Chapter 3, section 3.3.2 (McKenzie *et al.* 1999)). As a result, herbage DM yield of each species was re-ranked after N was applied. When N fertiliser was applied at medium or high rates, it was found that highest herbage DM yield was achieved from plantain and lowest herbage DM yield was achieved from red clover. This result was similar to previous chapters (Chapter 3, section 3.3.2) and previous experiments (Minneé *et al.* 2013). Differences in herbage DM yield between species was probably due to differences in thermal requirements (^{o}C days) and hormonal signalling altering above and below-ground partitioning of photosynthates in response to day-length and defoliation (Moot *et al.* 2000; Powell *et al.* 2007).

N fertiliser effect

The herbage DM yield response at any N fertiliser rate was greatest in plantain (20.6 kg DM/kg N applied in autumn and 43.7 kg DM/kg N applied in spring) and diploid perennial ryegrass (22.5 kg DM/kg N applied in autumn and 40.5 kg DM/kg N applied in spring), while lower responses occurred in chicory (12.4 kg DM/kg N applied in autumn and 26.6 kg DM/kg N applied in spring) and cocksfoot (9.8 kg DM/kg N applied in autumn and 29.9 kg DM/kg N applied in spring). This interesting result implies that both diploid perennial ryegrass and plantain need less N fertiliser to produce herbage DM yields similar to cocksfoot and chicory (King *et al.* 2012; Moore *et al.* 1991), reducing the N inputs and outputs into the farm system without hindering herbage DM yield. However, it could also imply that the high response forages, particularly in diploid perennial ryegrass, were deficient in N fertiliser at the medium N fertiliser rate. This was likely due to the cut and carry system with removal of herbage and underlines the importance of grazing animals to recycle and apply nutrients to pastures (Nevens and Rehuel 2003; Pincay-Figueroa *et al.* 2016; Wachendorf *et al.* 2004). In addition, it also highlights the need for legumes such as white clover which provide herbage N through biological N fixation to increase herbage DM yield in pastures (Chapman *et al.* 2017b; McKenzie *et al.* 1999; Mills and Moot 2010). Unsurprisingly, very low DM responses to N fertiliser occurred in legumes (-1.0 DM/kg N in autumn and 1.6 kg DM/kg N in spring, averaged over red and white clover). This was due to their ability to fix N through biological processes in the soil (McKenzie *et al.* 1999; Mills and Moot 2010; Moot *et al.* 2003).

4.4.2 Herbage N concentration

Forage effect

In the grasses and herbs, the herbage N concentration reached its maximum soon after N fertilisation and then decreased as regrowth interval increased. For example, after 1 week of N fertilisation, herbage N concentrations were 3.4 % in grasses and 4.1 % in herbs. However, by week 3, N concentrations had decreased to 3.2 % in grasses and 3.5 % in herbs and by week 4 herbages N concentrations were down to 2.8 % for grasses and 3.0 % for herbs. This is consistent with previous experiments using grass (Peyraud and Astigarraga 1998; Waghorn and Clark 2004; Wilman 1975) and herb (Lee *et al.* 2015) pastures. Herbage N concentration was lowest at the final harvest of 4 weeks. This is most likely due to N dilution rates in the plant cells as DM increased (Blaser 1964; Peyraud and Astigarraga 1998). This suggests, to reduce surplus herbage N intake in the herbage of livestock in autumn and spring, delaying grazing of herbs and grasses to 4 weeks had the greatest benefits, especially in diploid perennial ryegrass and plantain which contained the lowest N concentration. In the legumes, the N concentration of the herbage was the same regardless of regrowth interval in the autumn. This was due to their ability to fix N and demonstrates why they produced high herbage DM yields at the nil N fertiliser treatment. In spring however, legumes declined in herbage N concentration from weeks 3 to week 4 indicating that the plant may have been deficient in N. The reason for this may have been due to cooler temperatures in early spring which suppressed biological N fixation (Hoglund *et al.* 1974; Williams 1932; Young 1958).

At the final regrowth cut at 4 weeks, herbage N concentration was highest in legume species, intermediate in cocksfoot and chicory, and lowest in plantain and perennial ryegrass. In autumn, herbage N concentration was over 3 % (18 % crude protein), which exceed late lactation requirements for protein of dairy cows (AFRC 1993; Castillo *et al.* 2001; Pacheco and Waghorn 2008). This result indicates the risk of high urinary N losses, irrespective of species (Castillo *et al.* 2001). However, in spring, N concentrations were as low as 2.5 % N in the herbs and grasses indicating herbage N could be utilized more efficiently by livestock but may approach deficient levels. The reason for this is mostly likely due to a higher percentage of stem in the plant which contains lower concentrations of N compared to the leaf (Hoekstra *et al.* 2007). In addition, higher herbage DM yields may have caused a higher rate of N dilution and therefore, a lower concentration of N in the herbage (Peyraud and Astigarraga 1998).

N fertiliser effect

Lower N fertiliser rates in spring and autumn resulted in a lower herbage N concentration within herbage in all species except legumes. Although nil N fertiliser had the lowest herbage N concentration,

the compromise in yield is likely to offset herbage N concentration reductions. Therefore, a moderate N fertiliser rate resulted in lower N concentration compared with the high N rate.

The difference in herbage N concentrations between the N fertiliser treatments were notably higher at the beginning of the regrowth interval, compared to after 4 weeks of regrowth where N concentrations were similar between all N fertiliser rates. This suggests that using N fertiliser as a mitigation strategy to decrease herbage N concentration at the optimum time to graze (4 weeks) may be an ineffective way to reduce herbage N intake. However, earlier in the regrowth, herbage N concentration is more sensitive to N fertiliser rate thus, lower N fertiliser as a management strategy could be used if grazing the forages earlier e.g. at 2 or 3 weeks after regrowth in the spring and autumn.

The unresponsiveness of legumes to N fertiliser has been found in the previous chapter (Chapter 3, section 3.3.3) and reflects fact that legume herbage N concentration is unresponsive to N fertiliser. This is because of their ability to fix N in the soil, which supplies N for uptake into the plant, regardless of the N fertiliser rate (Clement *et al.* 2016).

4.4.3 Herbage WSC:CP ratio

Although herbage N concentration is an important driver of urinary N losses through determining herbage N intake by animals, other factors have also been recognised as influencing N concentration in the urine. One of these is an increase in supply of energy (WSC) relative to CP intake in herbage as a means to increase the capture of N in the rumen and reduce N excretion with consequent environmental benefits (Rasmussen *et al.* 2009). In a review of the effect of WSC, Edwards *et al.* (2007) proposed a WSC:CP ratio value of above 0.7 was the critical point to improve nitrogen use efficiency (NUE).

Forage effect

The WSC:CP ratio was found it be highest after 4 weeks of regrowth, in all the species, except red clover which was unaffected by regrowth interval. The reasons for a higher WSC:CP ratio after 4 weeks is likely due to a greater proportion of stem which is lower in N concertation (Hoekstra *et al.* 2007) and the plants reaching their threshold for replenishment of WSC reserves (Fulkerson and Slack 1994; Rawnsley *et al.* 2002).

When comparing forages, the WSC:CP ratio was lowest in legumes because of their ability to fix N which caused a higher CP concentration of the forage and offset the WSC:CP ratio (Edwards et al. 2007). Cocksfoot was also low in WSC:CP ratio because of high CP concentrations in the herbage as well as lower WSC concentrations offset the WSC:CP ratio. The reason for a low WSC:CP ratio in cocksfoot may have been due to the length of regrowth interval. Cocksfoot needs a longer regrowth interval than perennial ryegrass to replenish its WSC reserves (Rawnsley *et al.* 2002). As all grasses

were cut at the optimum harvest time for perennial ryegrass, WSC reserves in cocksfoot may have not been adequately replenished, resulting in lower WSC concentrations in cocksfoot (12 % of DM) compared to perennial ryegrass (23.5 % of DM) at week 4. This caused significantly lower WSC:CP ratios in the current trial of 0.4 in autumn, and 1.1 in spring at the fourth regrowth interval, averaged over the N fertiliser rates. The WSC:CP ratio was highest in diploid perennial ryegrass (1.0 in autumn and 2.3 in spring) because of low N concentration and indicates that, compared to other forages, it may lead to reduced N excretion. However, recent research feeding herbs to dairy cows found lower urinary N losses compared to when perennial ryegrass-white clover was fed (Box et al. 2016; Totty et al. 2013), despite low the WSC:CP ratios in herbs. These findings indicate that other chemical interactions in herbs may be involved in rumen N absorption, leading to lower N excretion (Box et al. 2016; Totty et al. 2013). The WSC:CP ratio was lower in herbs than perennial ryegrass at week 4 in both spring (1.31 vs 2.33, respectively) and autumn (0.89 vs 0.99, respectively). However, other plant compounds such as pectin (Barry 1998), acteoside (Navarrete et al. 2016) and sodium (Cheng et al. 2017) have higher concentrations in herbs and therefore suggests that factors other than herbage N intake may drive lower N excretion in livestock grazing pasture containing herbs (Stewart 1996; Tamura and Nishibe 2002).

N fertiliser effect

The WSC:CP ratio was affected by N fertiliser rate in diploid perennial ryegrass and cocksfoot. However, the WSC:CP ratio was unaffected by N fertiliser rate in the herb and legume forages. The WSC:CP ratio was highest in both grass forages at the nil N fertiliser rate and decreased as N fertiliser rate increased. This result was similar to previous experiments by Waite (1958), Bryant *et al.* (2012) and Loaiza *et al.* (2016) and reflects the increasing N concentration as N fertiliser increases, offsetting the balance between WSC and CP in perennial ryegrass. Adding to the balance is the decline in WSC caused by increased herbage DM yield in response to N fertiliser. This resulted in a dilution effect in the plant as cells got bigger (Coblentz *et al.* 2017; Van Soest 1994). Thus, applying higher rates of N fertiliser may decrease the WSC:CP ratio in the herbage and increase the rate of N lost in the urine. This is because of insufficient supply of energy for rumen microbes to offset N concentration in the animal's diet.

4.4.4 Herbage DOMD

Although using alternative pastures and extending regrowth interval could be a strategy to reduce N losses from farm systems, it is important to ensure quality of the pasture is high as to not impede animal production and farm profitability.

The energy value (DOMD) of the herbage did not decline over the regrowth interval in any of the species and remained high after 4 weeks (> 71 % DOMD). For example, the herbs increased from 72.7 % DOMD at week 1 to 76.0 % DOMD at week 4 in the spring and 69.7 % DOMD at week 1 to 79.3 %

DOMD at week 4 in the autumn. In addition, DOMD of grasses increased from 70.8 % at week 1 to 74.5 % at week 4 in the spring and 68.5 % at week 1 to 73.6 % at week 4 in the autumn. Finally, the DOMD of legumes was similar in spring (79.3 % at week 1 and 78.9 % at week 4) and increased from 74.3 % at week 1 to 77.3 % at week 4 in the autumn. This result was not expected as it was thought DOMD would generally decrease with regrowth interval because of a larger proportion of stem and dead matter as regrowth increased (Buxton 1996; Rawnsley *et al.* 2002). The reason for a high DOMD of 71 % or above, throughout the regrowth interval, was probably due to the time of year and the proportion of leaf in the herbage sampled above 4 cm. It has previously been found grasses harvested before the fourth leaf stage are higher in DOMD due to no leaf senescence (Donaghy and Fulkerson 1998). Leaf appearance interval (time taken for one leaf to fully expand) is influenced predominantly by temperature (Silsbury 1970) and soil moisture availability (Van Loo 1992). The current experiments were concluded in autumn and spring when cooler temperatures occurred and consequently, leaf appearance rate was slower. As a result, at week 4, the grasses were at the third leaf stage and leaf senescence did not occur. These results demonstrate that delaying regrowth to 4 weeks in autumn as a management strategy to reduce N losses, may not affect herbage quality and therefore farm production goals.

4.5 Conclusions and implications

The main implications drawn from this experiment are:

- Delaying grazing of grasses and herbs to 4 weeks in autumn increases herbage DM yield and WSC:CP ratios whilst reducing herbage N concentration with no detectable change in herbage quality as measured by DOMD.

- In autumn, the herbage N concentration of all forages was over 3 % N which exceeds animal N requirements and reflects the risk of high urinary N losses irrespective of species (Castillo *et al.* 2001). Regrowth management had little effect on N concentration at this time of year. Thus, alternative management strategies (e.g. supplementation and off paddock grazing) may be required to decrease N losses.

- In spring, herbage N concentration of both diploid perennial ryegrass and plantain did not exceed animal N requirements (Castillo *et al.* 2001) and therefore could be used as a low N diet for animals. However, at this time of year N losses would be low and reducing dietary intake of N to decrease nitrate leaching in farming systems, may be a limited strategy.

Chapter 5

Effects of nitrogen fertiliser rate on herbage DM yield, nitrogen concentration and nitrogen response rates of alternative pasture forages grown under glasshouse conditions

5.1 Introduction

Regional councils have brought in limits to restrict nitrate leaching, which may come with a risk to farm production and profit (Bryant *et al.* 2007). Therefore, approaches are needed to achieve these reductions whilst still maintaining or improving profitability.

In previous chapters, forages were identified that were high yielding and low in herbage nitrogen (N). For example, in Chapter 3, averaged over the two years plantain and prairie grass produced high annual herbage DM yields of 8,523 kg DM/ha/yr and 10,145 kg DM/ha/yr, respectively, with lower herbage N concentrations (2.8% N and 2.5 %N) compared with other forages such as white clover (9,922 kg DM/ha/yr, 4.5 % N). In addition, in Chapter 4, after 4 weeks of regrowth, plantain and perennial ryegrass produced high DM yields (1,544 kg DM/ha and 1,146 kg DM/ha, respectively) with low herbage N concentrations (2.8 % N and 2.5 % N, respectively) compared with white clover (1,325 kg DM/ha, 4.7 % N). This means they have the potential to provide animals with a low N diet, reducing urinary N whilst still maintaining high herbage dry matter (DM) yields for farmer profit. In addition, forages that were more efficient at utilising N fertiliser through higher DM response rates such as Italian ryegrass and prairie grass (29.2 and 21.6 kg DM/kg N applied, respectively, Chapter 3, averaged over the two years) and plantain and perennial ryegrass (32.2 and 31.5 kg DM/kg N applied, respectively, Chapter 4, averaged over the two years) are more sought after. This is because they demanded a lower rate of N fertiliser to obtain similar herbage DM yields to the other forages investigated in the experiment which equates to lower amounts of N circulating the farm system and lower N losses (Chapman *et al.* 2014; Whitehead 2000). This result suggests plantain and perennial ryegrass could reduce the risk of surplus N from fertiliser in the farm system.

Herbage DM yield responses to N fertiliser can often be described using a Mitscherlich type response curve, where eventually plants reach a maximum herbage DM yield potential and slow in plant growth (Chapman *et al.* 2014; Whitehead 2000). After this point, the plant N pool becomes saturated and plants may then take up 'luxury' amounts of N that is not needed, equating to excess N in the plant (Smith *et al.* 1985).

In Chapter 3, it was uncertain whether maximum herbage DM yield of forages were reached and there were no soil measurements to indicate whether plants started taking up luxury amounts of N from the soil or not. High herbage DM responses to N of 16 – 21 kg DM/kg N in the grasses and 14 kg DM/kg N in plantain at the highest N fertiliser rate of 450 kg N/ha/year in Chapter 3 suggests the forages did not reach maximum herbage DM yield and herbage DM yield continued to increase linearly, not tapering off as expected (Chapman *et al.* 2014; Smith *et al.* 1985; Whitehead 2000).

Therefore, the objective of this experiment was to determine the herbage N concentration and N fertiliser requirements in diploid perennial ryegrass, high sugar perennial ryegrass, Italian ryegrass, cocksfoot, prairie grass, chicory and plantain at maximum herbage DM yield under glasshouse conditions over a six-month summer and autumn period.

5.2 Materials and methods

5.2.1 Experimental site and design

The soil used was a free-draining Templeton fine sandy loam (Immature Pallic soil; (Hewitt 2010)). Soil was collected (0 – 150 mm horizon) from the Lincoln University Research Dairy Farm (LURDF), Canterbury, New Zealand (43°64'S, 172°46'E) on 3 September 2015. The soil was passed through a 13 mm sieve removing all plant material and then left to air dry until 11 September. Soil nutrient sampling was conducted before the experiment. Based on the results (Table 5.1), pots were fertilised with 0.4 g P ($Ca(H_2PO_4)_2$), 0.24 g S ($CaSO_4$) and 1.5 g K (KCL) to ensure nutrients were not limiting to the forages under investigation. The nutrients were added to 1.62 kg of air-dried soil and then mixed thoroughly. The soil was then lightly packed into 2 L pots with saucers to prevent drainage.

Table 5.1 Soil nutrient sampling prior to experiment of Templeton fine sandy loam (Immature Pallic soil; (Hewitt 2010)) from LURDF, Canterbury, New Zealand.

pH	Olsen P	Sulphate super	K	Ca	Mg	Na	CEC	Anaerobic mineralisable N*
	(mg/L)	(mg/kg)	----------(me/100g)----------					(kg/ha)
5.8	26	4	0.54	7.1	0.58	0.16	13	58

* Measured by anaerobic incubation (Keeney and Bremner 1966).

The pots were then moved to an unheated glasshouse at the Lincoln University glasshouse facility and arranged on tables in a split-plot, factorial design, with 7 forages as the main plot treatments, 7 N fertiliser rates as subplot treatments, to align with the plot experiment (Chapter 3), and four replicates. The forages were selected for this experiment because they were either high in herbage DM yield (plantain, prairie grass), low in herbage N concentration (prairie grass, high sugar grass, Italian ryegrass, diploid perennial ryegrass), or responded differently than expected in Chapters 3 and 4. For example, cocksfoot and chicory were higher in N concentration than expected. The forages evaluated were: diploid perennial ryegrass, high sugar perennial ryegrass, cocksfoot, Italian ryegrass, prairie grass, chicory and plantain. Cultivars of the forages can be found in Table 5.2. The total N fertiliser rates applied over the 134-day period were 0, 2.5, 5, 10, 17.5, 25 and 40 g N/m^2) (

Table 5.3). These rates equated to 0, 50, 100, 200, 350, 500 and 800 kg N/ha/year, to mimic the treatments used in the previous chapters (Chapter 3, section 3.2.1, Chapter 4 section 4.2.1). The 800 kg N/ha/year treatment was included to achieve maximum herbage DM yields. Nitrogen fertiliser was applied at the start of the experiment and following each harvests as calcium ammonium nitrate (27 : 0 : 0 : 0; N : P : K : S), with the total N application rate split throughout the experiment. This is shown in

Table 5.3.

Table 5.2 Forage sown, functional group, scientific name, cultivar, sowing rate (kg/ha), 1000 seed weight (g) and sowing rate (seeds/pot).

Forage	Functional group	Scientific name	Cultivar	Sowing rate (kg/ha)	1000 seed weight (g)	Sowing rate (seeds/ pot)

Perennial ryegrass – late season flowering diploid	Grass	*Lolium perenne*	One-50 (AR37)	20	3.9	8
High sugar perennial ryegrass - late season flowering diploid	Grass	*Lolium perenne*	AberMagic (AR1)	18	2	15
Italian ryegrass - diploid	Grass	*Lolium multiflorum*	Tabu	25	2.4	17
Cocksfoot	Grass	*Dactylis glomerata*	Savvy	8	0.9	15
Prairie grass	Grass	*Bromus willdenowii*	Atom	25	11.5	4
Chicory	Herb	*Cichorium intybus*	Choice	8	1.2	11
Plantain	Herb	*Plantago lanceolata*	Tonic	10	2	8

Table 5.3 Total N applied in the experiment (19 weeks) and N applied per application for each N fertiliser rate.

Total N applied in experimental period*		**N applied per application**	
(g/m^2)	(kg/ha)	(g/m^2/application)	(kg/ha/application)
0.0	0	0.0	0
2.5	25	0.5	5
5.0	50	1.0	10
10.0	100	2.0	20
17.5	175	3.5	35
25.0	250	5.0	50
40.0	400	8.0	80

* Total N applied for period of 19 weeks (134 days), equivalent to 0, 50, 100, 200, 350, 500 and 800 kg N/ha/year.

The glasshouse temperature was maintained within the range of 14 – 28 °C with a mean air temperature of 19.6 °C for the duration of the experiment. Seeds were sown directly into the soil as monocultures on 29 September 2015 (early spring) using a 'grid' sowing pattern (10 mm by 10 mm spacing). The number of seeds per pot varied between each forage and were calculated using 1000 seed weight (g) and sowing rates (kg/ha) as shown in

Table 5.3. A small quantity of soil (2mm depth) was sprinkled over the seeds after sowing and gently packed down (Plate 5.1). Soon after germination, emerged plants were counted, and more seeds were sown if not enough plants had established per pot. The target seeding population was the same as the sowing rate per pot (Figure 5.3).

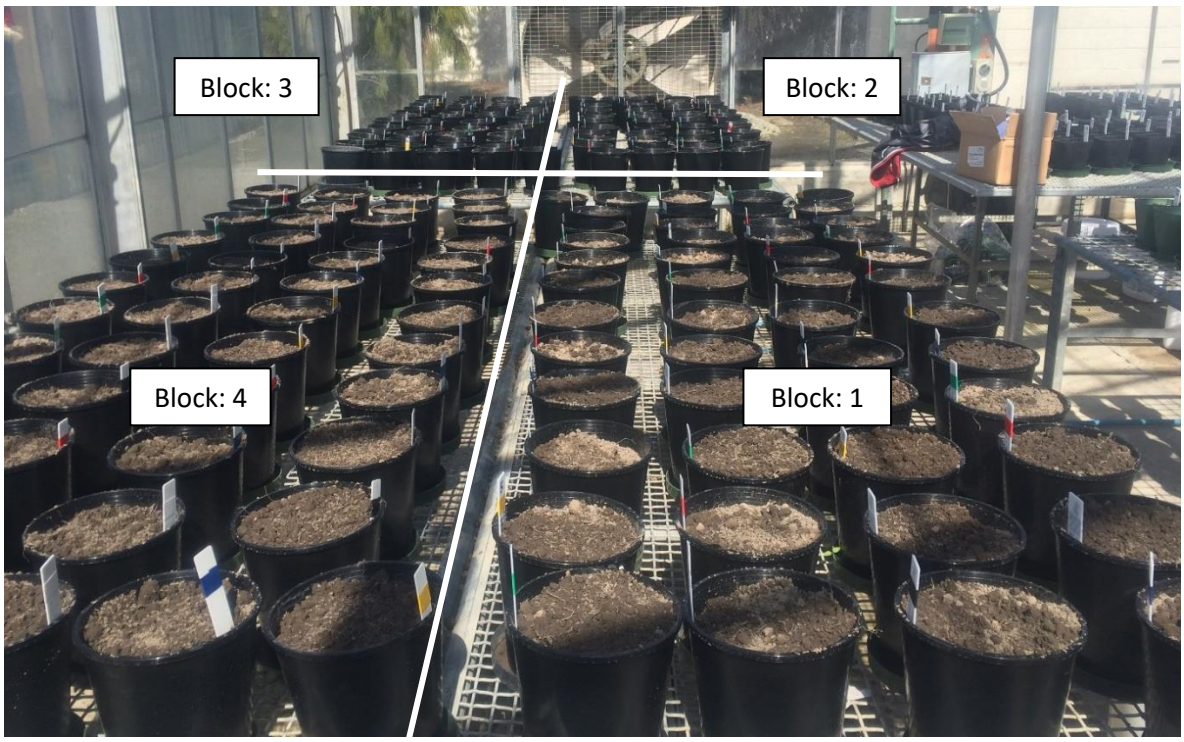

Plate 5.1 Pots arranged on tables as a split plot factorial design, with seven forages as the main plot treatments and seven N fertiliser rates as subplot treatments.

5.2.2 Management

The pots were watered using an automated irrigation system. An ECH_2TM soil moisture sensor was used to detect when irrigation was needed by measuring the dielectric constant which relates to water in the soil. A total of seven soil moisture sensors were placed in the pots, one for each N fertiliser treatment. When average soil moisture levels were below 24 % moisture, as read by the soil moisture sensors, a signal triggered the automated irrigation and water was applied at a rate of 11.1 ml per irrigation event. Often more than one irrigation event would occur in a day to ensure average soil moisture between the pots was maintained. The irrigation was supplied on two different schemes which were low and high; the low irrigation scheme supplied water to the 0-10 g N/m^2 treatment pots and the high irrigation scheme supplied water to the 17.5-40 g N/m^2 treatment pots. This ensured high yielding plants were not restricted due to water stress and low yielding plants were not over watered and became waterlogged.

Plants were allowed to establish in the absence of defoliation for 65 days after sowing (9 weeks post germination, Plate 5.2). The experiment started after the first harvest on 30 November 2015 and ended 134 days later on 12 April 2016. Five herbage harvests were conducted within the experimental period which were 4, 8, 12, 15 and 19 weeks after the first defoliation. Nitrogen fertiliser treatments were applied in five evenly split applications over the experimental period (19 weeks). The first application of N was applied to the pots after the first defoliation on 30 November 2015, and from then after on 30 December 2015, 26 January 2016, 19 February 2016 and 18 March 2016.

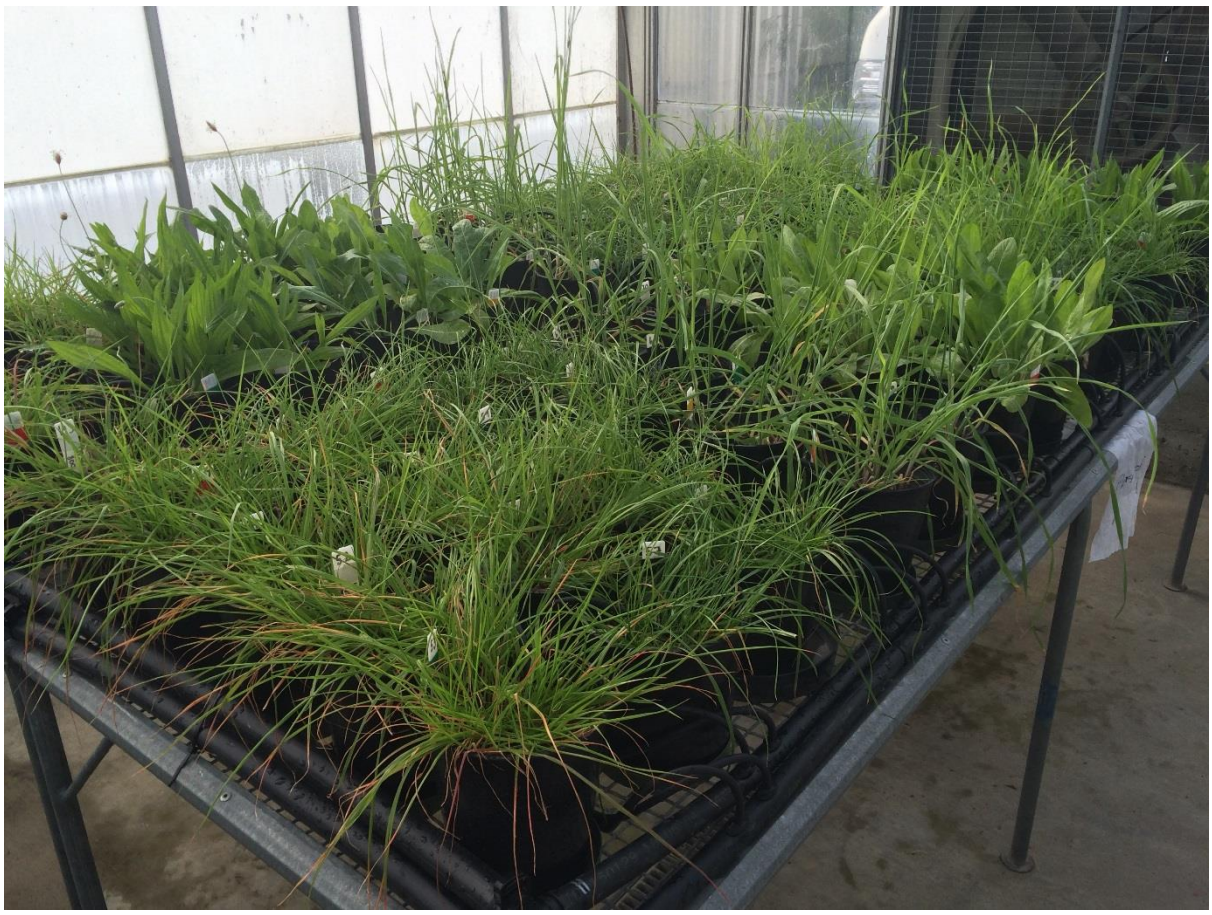

Plate 5.2: Established plants 65 days after sowing (9 weeks post germination), prior to first harvest.

5.2.3 Herbage and soil measurements

Herbage was defoliated by cutting with scissors to 4 cm above ground level to mimic grazing height. Herbage was oven dried in 60 ^{o}C for 48 hours (Adesogan *et al.* 2000) and weighed for herbage DM yield. Following weighing, samples were ground through a 1 mm sieve with a M200 rotor mill (Retsch Inc., Newtown, Pennsylvania, USA) and scanned by near-infrared spectrophotometry (NIRS, NIRSystems 5000, Foss, Maryland, USA) to determine crude protein (CP) concentration, based on calibrations derived on the experimental herbages in Chapter 3 (section 3.3.3). Nitrogen concentration was calculated by dividing CP by 6.25. At final harvest 50 g soil samples were taken from each pot and analysed for anaerobic mineralisable N.

Accumulated herbage DM yield was calculated from the start of the experiment on the 30 November 2015 until the end on 12 April 2016. Thus, total herbage DM yield represented herbage harvested over 134 days of growth. Herbage N concentration was averaged over the five harvests and total N uptake was calculated using the accumulated herbage DM yield and average herbage N concentration over the experimental period. Accumulated herbage DM yield and total N applied were used to calculate the DM responses to N by calculating the extra growth from added N fertiliser and dividing it by the amount of N fertiliser added.

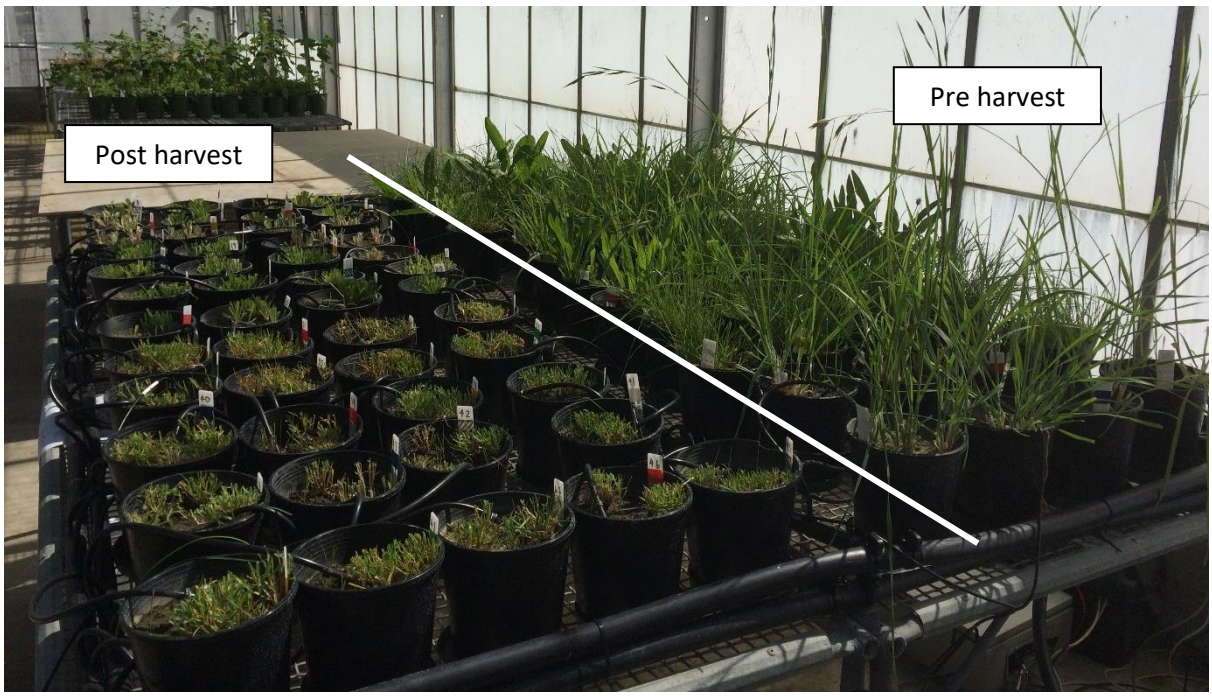

Plate 5.3: Pre and post harvest of plants on 19 February 2016.

Glasshouse temperatures were measured using a computer thermometer which monitored the air temperature every two hours. This was averaged on a monthly basis to generate the maximum and minimum monthly air temperature. Irrigation was measured using the computer automated irrigation system that applied the water to the pots.

5.2.4 **Statistical analyses**

The effects of N fertiliser rate on herbage DM yield, N response rates (kg DM grown per kg N applied), herbage N concentration, plant N uptake and anaerobic mineralisable N was analysed by an analysis of variance (ANOVA) with split plot design using GenStat Version 16 (VSN International Ltd) where forages were the main plot and N rates were the sub plot. The model included N rate, forages and the N rate x forage interaction. Highly significant ($P<0.001$) interaction effects were observed in N uptake results and therefore regression analysis and curve fitting was undertaken for all forages individually in order to better interpret the response of forage to N rate.

5.3 Results

5.3.1 Air temperature and irrigation

Average air temperature in the glasshouse was 19.4 °C from when the plants were sown on 29 September 2015 to the end of the experiment on 12 April 2016 and 19.6 °C over the course of the experiment from 30 November 2015 to 12 April 2016 (Figure 5.1). This exceeded the value of typical outdoor air temperatures by 6.1 and 6.3 °C if plants were grown in the field. Temperatures were highest in February, where the maximum daily temperature reached 27.5 °C, and lowest in September and April where temperatures averaged 18.3 and 18.6 °C, respectively.

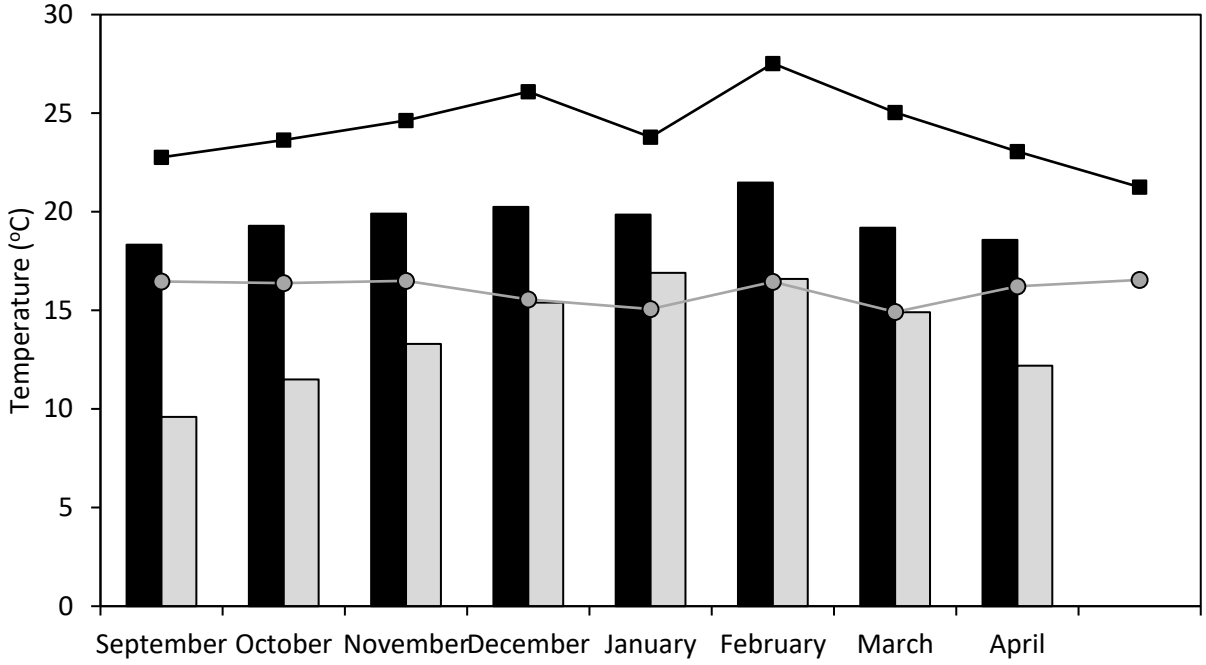

Figure 5.1 Average monthly air temperatures (black) and maximum (—■—) and minimum (—●—) monthly air temperatures of glasshouse over experimental period. Outside air temperature (grey) has been calculated using long term averages between 1981 and 2010.

Total automated irrigation applied to the low irrigation pots was 223 mm (11 December 2016 to 12 April 2016, Figure 5.2). This was 103 mm lower than the high irrigation pots which received 326 mm total irrigation.

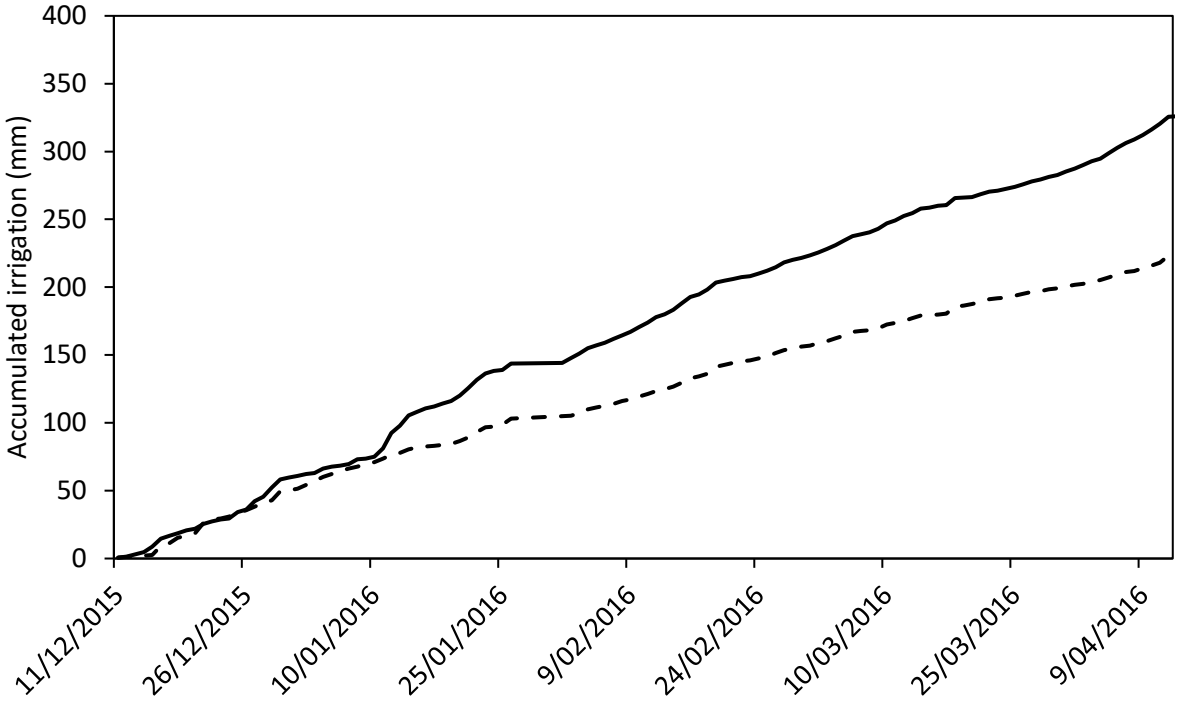

Figure 5.2 Accumulated irrigation for low irrigation scheme pots (- - -) and high irrigation scheme pots (—) from 11 December 2015 to 12 April 2016.

5.3.2 Herbage DM yield

Forages differed by up to 18 % in accumulated herbage DM yield (P<0.001) and, after 19 weeks, herbage DM yield was highest in prairie grass (456 g DM/m^2) and lowest in diploid perennial ryegrass (373 g DM/m^2) (Table 5.4). Accumulated herbage DM yield increased as N fertiliser rate increased in all the forages. Averaged across species this was from 106 g DM/m^2 to 941 g DM/m^2 (P<0.001, Table 5.4, Figure 5.3). There was no interaction in herbage DM yield between forage and N fertiliser rate, thus maximum yield occurred at the highest N fertiliser rate of 40 g N/m^2 in all the forages as shown in Plate 5.4.

Table 5.4 Accumulated herbage DM yield, herbage N concentration, total herbage N uptake, DM response rates to N and soil mineralisable N of seven pasture forages grown under glasshouse conditions with increasing rates of N fertiliser rates ranging from 0 – 40 g/m^2.

Forage	Total N treatment (g/m^2)	Accumulated DM yield (g DM/m^2)	DM response rates (g DM/g N)	N conc. (% of DM)	Total N uptake (g N/m^2)	Soil mineralisable N (g/m^2)
Chicory		433	20.5	2.0	9.3	6.1
Plantain		451	22.7	1.9	9.5	5.7
Cocksfoot		452	24.6	2.2	10.1	7.9
HSG		419	19.8	2.2	9.7	8.5
Italian RG		388	19.1	2.0	8.3	8.2
Prairie grass		456	24.0	1.9	9.1	7.6
Diploid PRG		373	20.3	2.1	8.1	7.9
Forage LSD		**29.4**	**7.43**	**0.1**	**0.58**	**1.22**
	0	106	-	1.9	2.0	7.6
	2.5	154	19.2	1.9	3.0	7.5
	5	213	21.3	1.9	4.1	7.5
	10	275	16.9	2.0	5.6	6.7
	17.5	585	27.4	1.9	11.3	7.0
	25	700	23.8	2.1	14.6	7.2
	40	941	20.9	2.5	23.6	8.6
	N rate LSD	**32.9**	**3.35**	**0.1**	**0.66**	**0.81**
F Pr.	Forage (F)	***	NS	***	***	***
	N rate (N)	***	***	***	***	***
	F x N	NS	NS	NS	*	NS

Conc., concentration; HSG, high sugar perennial ryegrass; Italian RG, Italian ryegrass, Diploid PRG, diploid perennial ryegrass; NS, not significant.

*P≤0.05

**P<0.01

***P<0.001

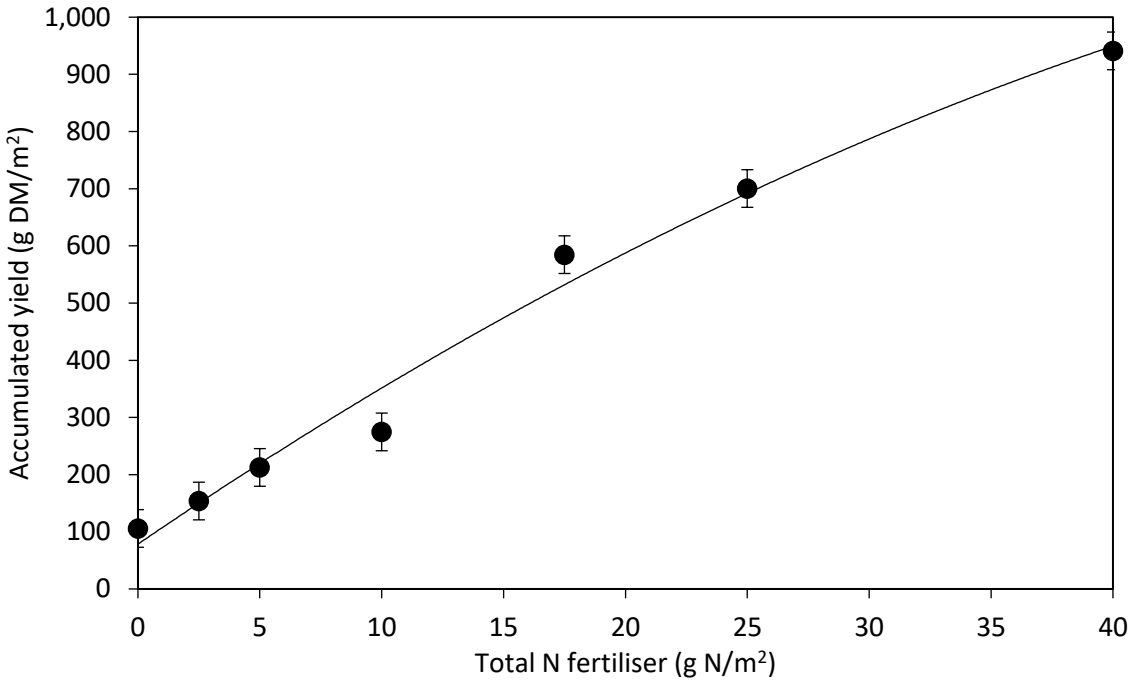

Figure 5.3 Effect of increasing N fertiliser rates ranging from 0 – 40 g/m^2 on accumulated herbage DM yield averaged over the seven forages grown under glasshouse conditions. Data are means values ± LSD. Regression is as follows: y = -0.1841x^2 + 29.144x + 78.379 (R^2 = 0.98).


(a)
0 g N/m²
2.5 g N/m²
5 g N/m²
10 g N/m²
17.5 g N/m²
25 g N/m²
40 g N/m²
(b)
0 g N/m²
2.5 g N/m²
5 g N/m²
10 g N/m²
17.5 g N/m²
25 g N/m²
40 g N/m²
(c)
0 g N/m²
2.5 g N/m²
5 g N/m²
10 g N/m²
17.5 g N/m²
25 g N/m²
40 g N/m²
(d)
0 g N/m²
2.5 g N/m²
5 g N/m²
10 g N/m²
17.5 g N/m²
25 g N/m²
40 g N/m²
(e)
0 g N/m²
2.5 g N/m²
5 g N/m²
10 g N/m²
17.5 g N/m²
25 g N/m²
40 g N/m²

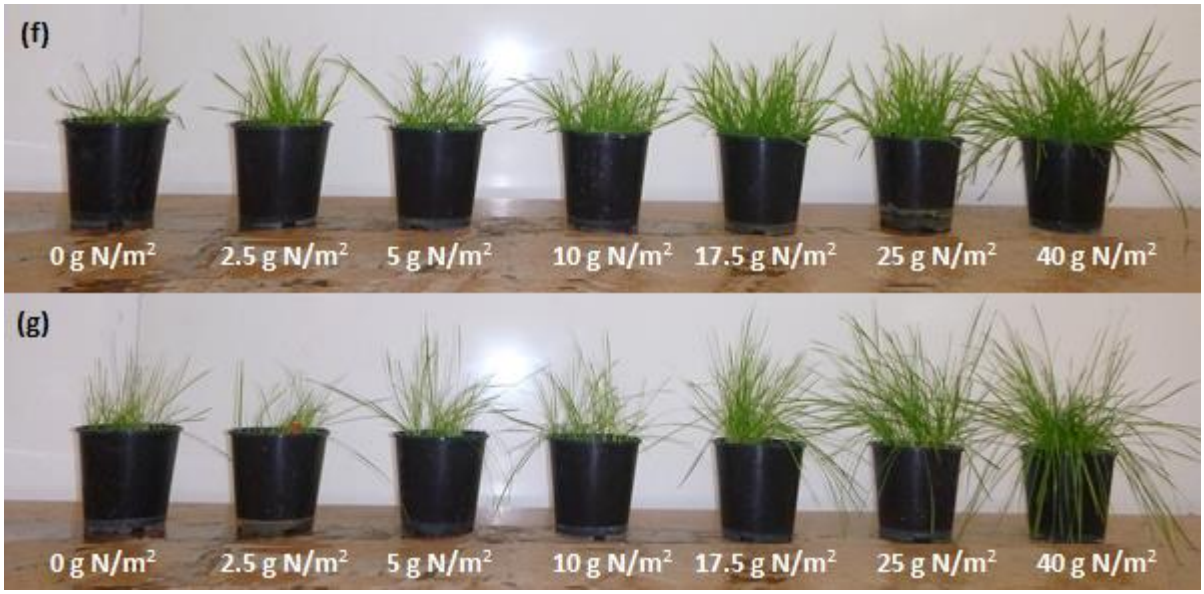

Plate 5.4 Pre harvest of forages (a) plantain, (b) chicory, (c) prairie grass, (d) Italian ryegrass, (e) diploid perennial ryegrass, (f) cocksfoot and high sugar perennial ryegrass (g) before the final harvest with increasing total N fertiliser rates (0-40 g N/m^2).

5.3.3 Herbage DM yield response

The herbage DM response rates were not different between the forages, with an average response rate of 21.6 g DM per g N applied (Table 5.4). However, as N fertiliser rate increased, response rates increased from the 2.5 g N/m^2 fertiliser rate (19.2 g DM/g N) to the 17.5 g N/m^2 fertiliser rate (27.4 g DM/g N), but then decreased after this to 20.9 g DM/g N at the highest N fertiliser rate (40 g N/m^2) (Table 5.4, Figure 5.4).

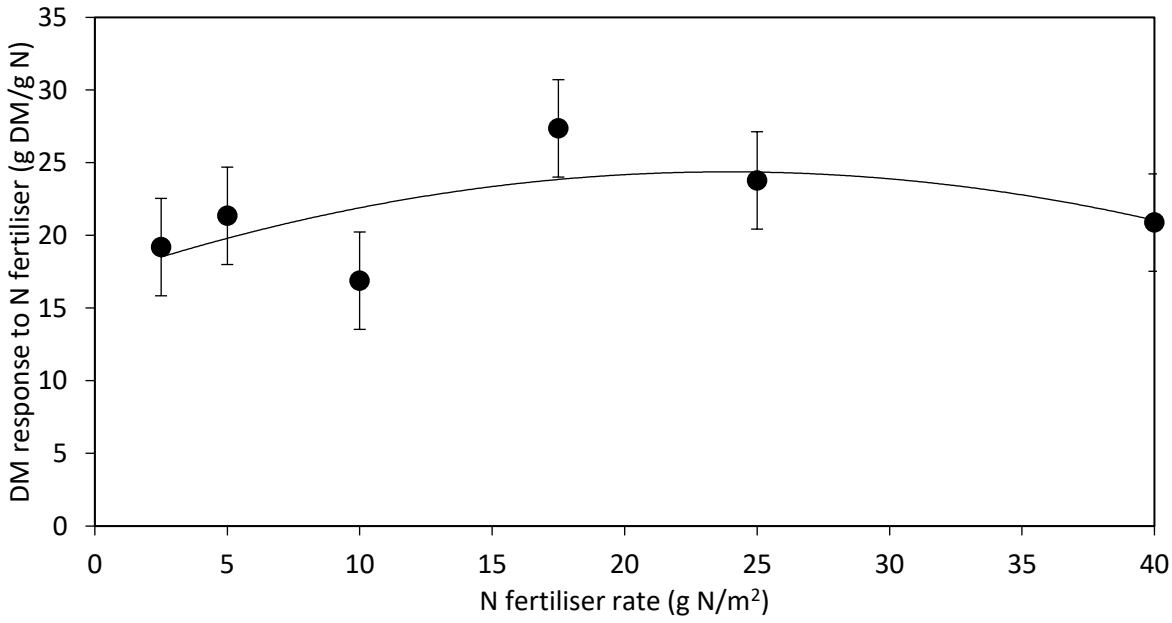

Figure 5.4 Effect of increasing total N fertiliser rates ranging from 0 – 40 g/m^2 on DM response to N fertiliser averaged over the seven forages grown under glasshouse conditions. Data are means values ± LSD. Regression is as follows: y = -0.0128x^2 + 0.6124x + 17.057 (R^2 = 0.39).

5.3.4 Herbage N concentration and uptake

Mean herbage N concentration was highest in the high sugar perennial ryegrass and cocksfoot (2.2 % N), intermediate in chicory (2.1 % N), diploid perennial ryegrass (2.1 % N) and Italian ryegrass (2.0 % N) and lowest in prairie grass and plantain (1.9 % N, P<0.001, Table 5.4). There was no interaction between forages and N fertiliser rate. However, herbage N concentration increased significantly (P<0.001) when N applications equal to or more than 10.0 g N/m^2 were applied to all the forages, demonstrating a increasing quadratic response to N fertiliser treatment (Table 5.4, Figure 5.5). This was especially significant at the highest N fertiliser rate which increased in herbage N concentration to 2.5 % N.

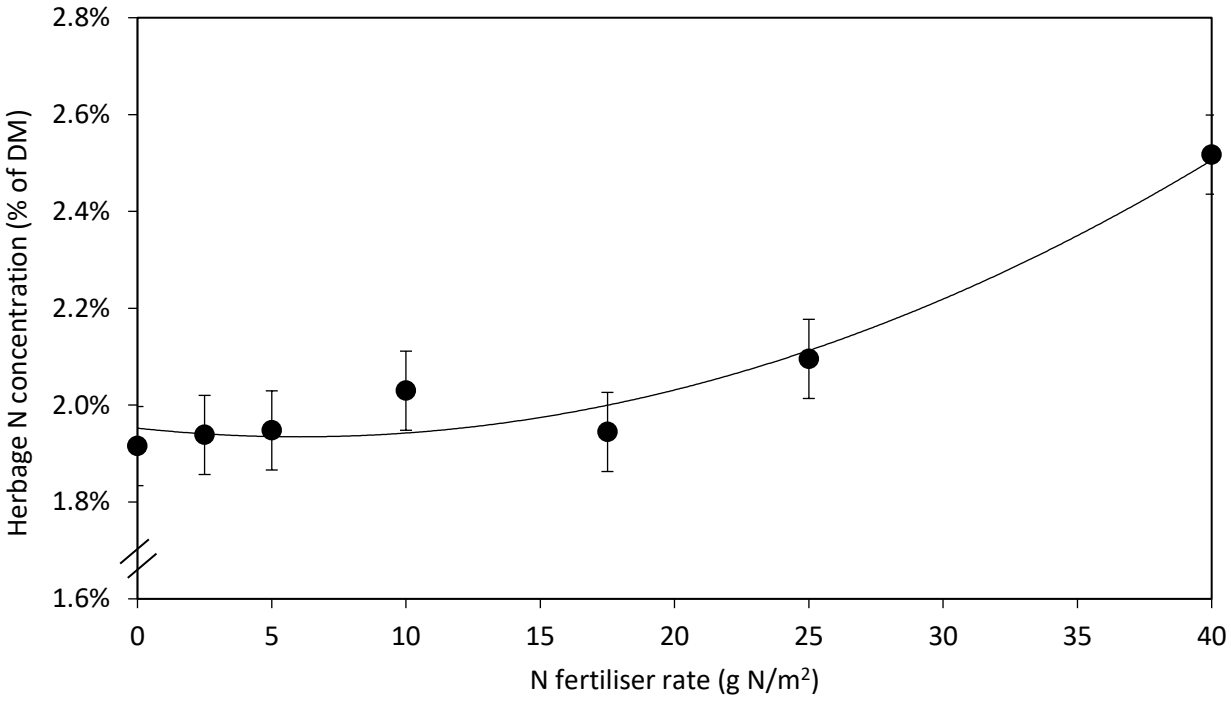

Figure 5.5 Effect of increasing N fertiliser rates ranging from 0 – 40 g/m^2 on herbage N concentration averaged over the seven forages grown under glasshouse conditions. Data are means values ± LSD. Regression is as follows: y = 5E-06x^2 - 6E-05x + 0.0195 (R^2 = 0.95).

An interaction between N fertiliser and forage was shown in the total N uptake of the forages (P≤0.05, Figure 5.6). Whilst at the low N fertiliser rate only a small difference of 0.7 g N/m^2 was shown between the forages, at the high N fertiliser rates, a difference of 4 g N/m^2 was shown suggesting total N uptake was different between the forages. N uptake in cocksfoot and high sugar perennial ryegrass were the most responsive to N fertiliser with total N uptakes of 25.5 g N/m^2 at the highest N fertiliser rate (Table 5.4, Figure 5.6). However, diploid perennial ryegrass was least responsive with N uptake values of 20.8 g N/m^2 at the highest N fertiliser rate. The effect of N fertiliser rate in both chicory and plantain was intermediate with a total N uptake of 24.5 g N/m^2 (chicory) and 23.8 g N/m^2 (plantain) at the highest N rate.

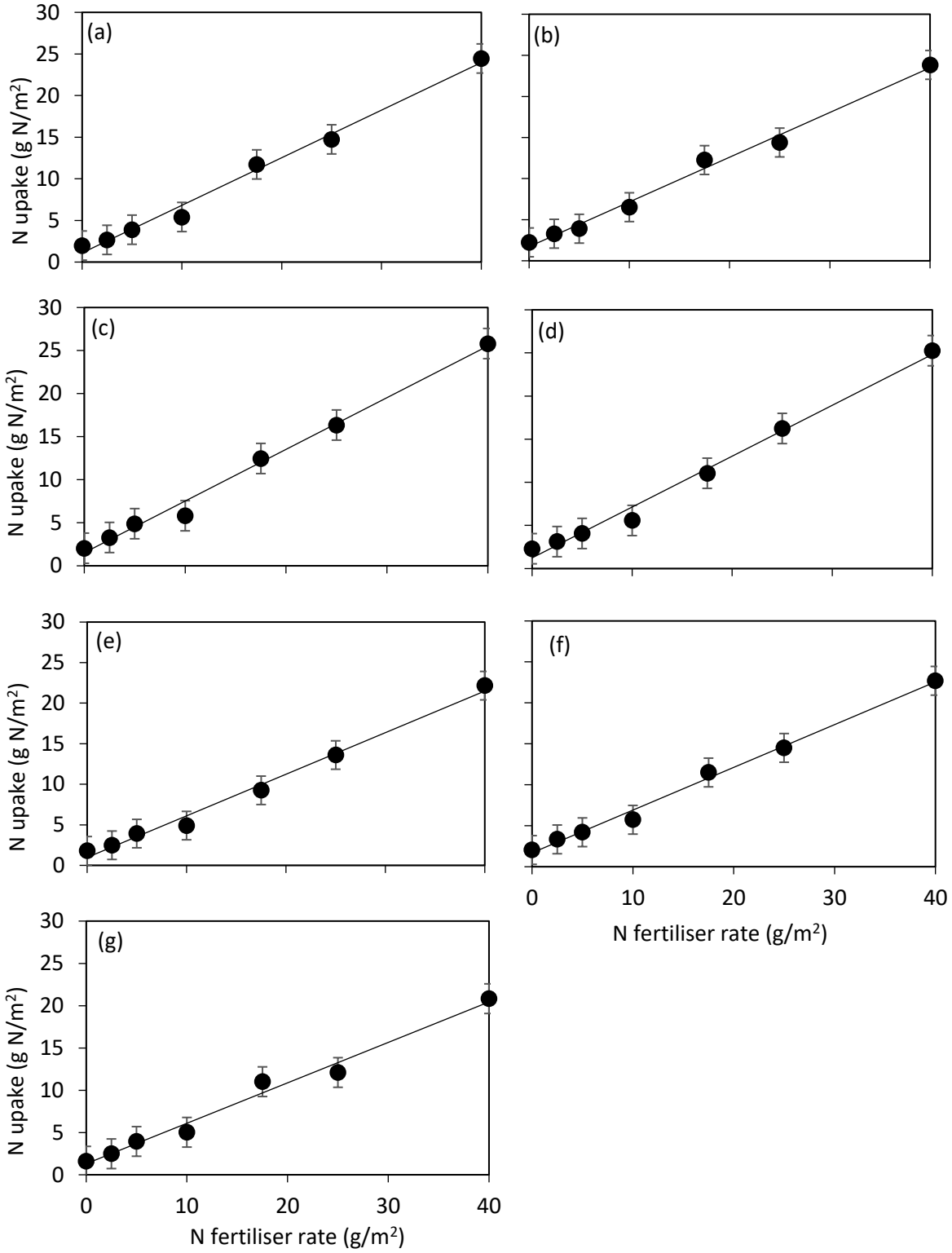

Figure 5.6 Effect of increasing N fertiliser rates (g/m²) ranging from 0 – 40 g/m² on total N uptake of six forages: chicory (a), plantain (b), cocksfoot (c), high sugar perennial ryegrass (d), Italian ryegrass (e), prairie grass (f) and diploid perennial ryegrass (g) grown under glasshouse conditions. Data are means values ± LSD. Regression of each forage is as follows: chicory y = 0.5717x + 1.1019 (R^2 = 0.99), plantain y = 0.5442x + 1.7178 (R^2 = 0.99), cocksfoot y = 0.6003x + 1.5205 (R^2 = 0.99), high sugar perennial ryegrass y = 0.5898x + 1.2359 (R^2 = 0.99), Italian ryegrass y = 0.5129x + 0.9755 (R^2 = 0.99), prairie grass y = 0.5217x + 1.6937 (R^2 = 0.99) and diploid perennial ryegrass y = 0.4782x + 1.3097 (R^2 = 0.98).

5.3.5 Soil mineralisable N

There was no interaction between N fertiliser and forage in soil mineralisable N. Soil mineralisable N in the herbs chicory and plantain were significantly (P<0.001) lower than the grasses (5.9 g N/m^2 vs 8.0 g N/m^2, Table 5.4). In addition, as N fertiliser rate increased, soil mineralisable N in all the forages decreased from the 0 g N fertiliser rate (7.6 g N/m^2) to the 10 g N fertiliser rate (6.7 g N/m^2) but then increased after this to 8.6 g N/m^2 (Table 5.4, Figure 5.7).

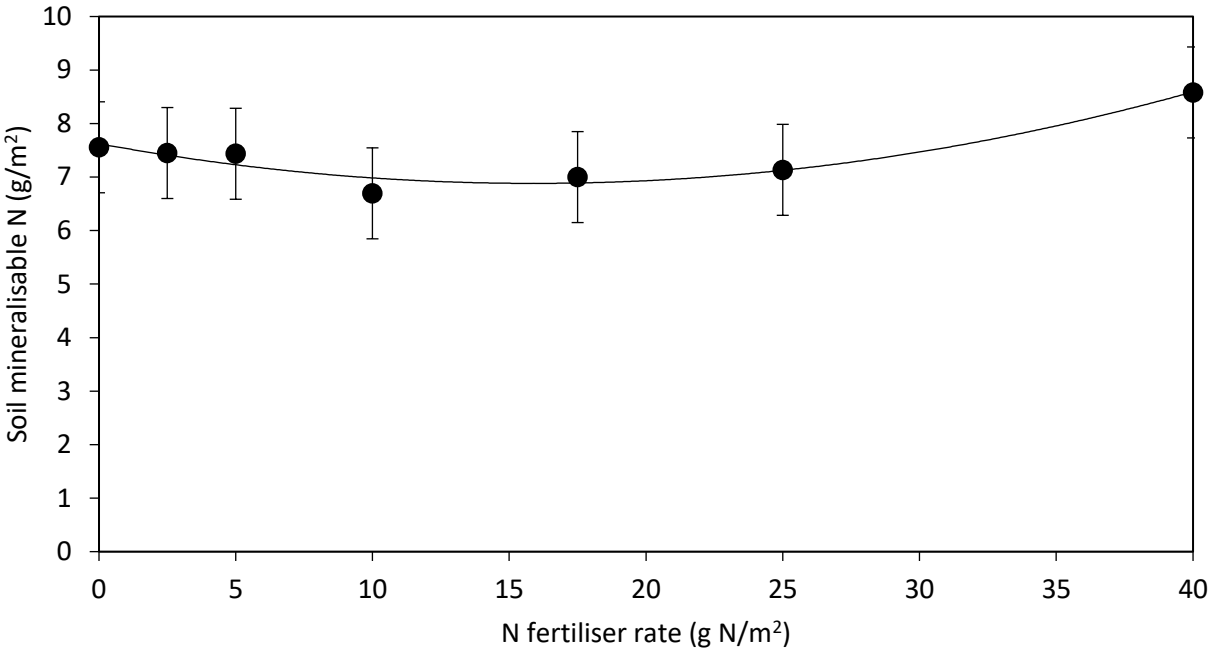

Figure 5.7 Effect of increasing N fertiliser rates ranging from 0 – 40 g/m^2 on residue soil mineralisable N at the end of the experiment averaged over the seven forages grown under glasshouse conditions. Data are means values ± LSD. Regression is as follows: $y = 0.0029x^2 - 0.0934x + 7.6385$ ($R^2 = 0.93$).

5.4 Discussion

5.4.1 Herbage DM yield and N response rate

Forage effect

Accumulated herbage DM yield was highest, average over the N fertiliser rates, in prairie grass (456 g/m^2), cocksfoot (453 g/m^2) and plantain (451 g/m^2), intermediate in chicory (433 g/m^2) and high sugar perennial ryegrass (419 g/m^2) and lowest in Italian ryegrass (388 g/m^2) and diploid perennial ryegrass (373 g/m^2). The high growth rates in prairie grass, at all fertiliser rates, were also found in Chapter 3, and may reflect its faster leaf appearance rate and larger leaves (Belton 1992; Hume 1991; Turner *et al.* 2006). The high herbage DM yield of cocksfoot compared other forages such as perennial ryegrass (373 g DM/m^2) and Italian ryegrass (388 g DM/m^2), potentially reflects the experimental conditions. Warmer temperatures in the glasshouse compared to outside (6.1 ^{o}C higher) is likely to have increased evapotranspiration and may have caused lower soil moisture in the pots. Although the soil moisture content in the pots was targeted at 24% soil moisture, the actual soil moisture levels fluctuated between 36 % soil moisture (0 g N/m^2) and 8 % soil moisture (40 g N/m^2 treatment). Cocksfoot is more drought tolerant due to its dehydration tolerance in surviving tissue and its ability to extract water from the roots at lower soil water potentials (Volaire and Lelievre 2001). Therefore, the herbage DM yield of cocksfoot is likely to have been less affected by the soil conditions and could be the reason for higher herbage DM yields (Barker *et al.* 1993; Kemp *et al.* 1999b). A deeper rooting system and the presence of the chemical aucubin in plantain may explain the higher accumulated herbage DM yield recorded for this forage. A more vigorous root may allow plantain to take up more water from further down the soil profile, therefore making it less prone to water stress. The presence of aucubin previously been found in plantain (Dietz *et al.* 2013), has been shown to have a inhibitory effect on soil N mineralisation meaning soil N is less likely to be converted into nitrate and easily leached. Instead it remains in the soil as ammonia providing plantain with a greater amount of readily available soil N which is easily taken up by the roots causing higher herbage DM yields.

Herbage DM yield was lowest in Italian ryegrass and diploid perennial ryegrass with values of 388 g/m^2 and 373 g/m^2, respectively, averaged over all N fertiliser rates. This result conflicts with the results of Chapter 3, which found Italian ryegrass had high herbage DM yield compared to the other grass forages. The reason for these lower herbage DM yields in these two species was possibly due to high temperatures in the daytime in summer which limited herbage DM yields of grasses with cool-season characteristics such as perennial and Italian ryegrass. Hunt and Field (1979) showed appearance rates of tillers per day in perennial ryegrass declined over 20 ^{o}C, and after 30 ^{o}C the death of leaves occurred. Considering this, with temperatures reaching maximum values of 27.5 ^{o}C in February and 25 ^{o}C in March, it is likely the perennial ryegrass forages were not able to tolerate the higher temperatures. This resulted in lower herbage DM yields in these forages, compared to more drought tolerant forages,

such as cocksfoot. In Chapter 3, Italian ryegrass had high herbage DM yield in early spring compared to other species because of its cool season growth traits. In addition, previous experiments (Malcolm *et al.* 2014; Moir *et al.* 2013) conducted in winter and early spring, have shown Italian ryegrass had high cool season growth compared to other forages such as perennial ryegrass, tall fescue and diverse pasture mixtures (perennial ryegrass/Italian ryegrass/white clover/red clover/chicory/plantain). The higher herbage DM yields have the potential to reduce N losses through greater plant N uptake from the soil. However, the current experiment did not include winter and early spring conditions, and this competitive advantage of Italian ryegrass many not have been represented. If the experiment was carried out over a longer period, different outcomes may have occurred.

N fertiliser effect

Increasing N fertiliser rates resulted in total accumulated herbage DM yields up to 941 g DM/m^2 at the highest N rate (40 g N/m^2) compared to the 106 g DM/m^2 at the lowest N fertiliser rate (0 g N/m^2 N), averaged over all the forages in spring and summer. This result was consistent with previous chapters (Chapter 3, section 3.4.1 and Chapter 4, section 4.3.2). Results from the current experiment also showed the soil N concentration of the treatments were highest at the high N fertiliser rates (8.6 g/m^2 at the 40 g N/m^2 N fertiliser rate vs 7.0 g N/m^2 at the 17.5 g N/m^2 N fertiliser rate). This means utilisation and efficiency of N within the plant is low suggesting the forages are slow at taking up luxury amounts of soil N, increasing the risk of N losses through leaching at the high N treatments.

Herbage DM response rates to N fertiliser in all the forages were highest at the medium (17.5 g N/m^2) N fertiliser rate (27.4 g DM/ g N), and lowest at the lower (0 and 10 g N/m^2) N fertiliser rates (19.2 and 16.9 g DM /g N) suggesting the forages were N deficient. Herbage DM response rates in the current experiment were high (21.6 g DM/g N) compared to previous pot and field experiments. For example, a pot trial by Moir *et al* (2013) showed perennial ryegrass had N response rates ranging between 3.7 and 7.3 g DM/g N. Furthermore, a field trial by Moir *et al.* (2003) showed perennial ryegrass had N response rates in Canterbury ranged between 5 and 14 g DM/g N in summer and 4 and 15 g DM/g N in autumn.

5.4.2 Herbage N concentration

Forage effect

Herbage N concentration, averaged over N fertiliser rates, was lowest in prairie grass and plantain (1.9 % of DM) and highest in cocksfoot and high sugar perennial ryegrass (2.2 % of DM). These herbage N concentrations were much lower than the previous field experiments (Chapter 3, section 3.3.3, Chapter 4 section 4.3.4) which showed herbage N concentrations, averaged over the N fertiliser rates and forages, ranged from 2.8 to 3.7 % N over late spring, summer and autumn. In addition, previous pot studies conducted in a glasshouse showed higher herbage N concentration values of 3.6, 3.7, 3.5

and 3.6 % N in cocksfoot, Italian ryegrass high sugar perennial ryegrass and diploid perennial ryegrass, respectively (Moir *et al.* 2013). The reason for the low herbage concentration is unclear but may reflect low soil mineralisable N at the beginning of this study (5.8 g N/m^2) compared to Moir *et al.* (2013) (16.0 g N/m^2). In addition, in the pot trial of Moir *et al* (2013), N herbage measurements were carried out through the winter when temperatures were lower (minimum of 10^{O}C at night). This resulted in lower herbage DM yields and cell division in the base of the plant, and therefore lower N dilution rates and higher N concentration in the plant (Hoekstra *et al.* 2007; Peyraud and Astigarraga 1998). As temperatures in the current experiment did not drop below 14.9 ^{o}C at night, this may have contributed to lower herbage N concentration in the plants through higher N dilution rates.

The lower herbage N concentration in prairie grass and plantain may be explained by higher herbage DM yields of these two species leading to N dilution in the herbage (Hoekstra *et al.* 2007; Peyraud and Astigarraga 1998). This explanation is consistent with previous chapters where, high yielding forages, for example, prairie grass and Italian ryegrass contained lower herbage N concentration (Chapter 3, section 3.3.3 and Chapter 4, 4.4.2). On the other hand, most forages with lower herbage DM yields had a higher N concentration due to less N dilution in the leaves. This was shown in high sugar perennial ryegrass and diploid perennial ryegrass which contained higher N concentrations of 2.2 % N and 2.1 % N in DM, respectively, and produced the lowest total herbage DM yields (396 g/m^2, averaged) compared to the other forages. Cocksfoot however, was the exception in that it contained higher amounts of herbage N (2.1 % N) and was also higher in herbage DM yield (453 g/m^2) compared to the other forages. This result was also shown in previous chapters (Chapter 3, section 3.3.3, Chapter 4, section 4.4.2) and may be explained by a lower proportion of water soluble carbohydrates in cocksfoot (data not shown) caused by a short regrowth interval which resulted in less dilution of herbage N and therefore a higher N concentration in the leaves (Rawnsley *et al.* 2002).This was shown in Chapters 3 and 4 where cocksfoot contains a lower herbage N concentration. This result indicates grazing cocksfoot later than diploid perennial ryegrass is better from an environmental perspective as it leads to a lower N intake. Consequently, harvesting all the forages at the same time may be a limitation in the pot experiment.

5.4.3 Soil Mineralisable N

Soil mineralisable N in the pots after the final harvest ranged from 5.7 g N/m^2 (plantain) to 8.5 g N/m^2 (high sugar perennial ryegrass) averaged over the N fertiliser rates. This was low compared to previous experiments such as Moir *et* al (2013) where it was found soil mineralisable N was 16.0 g N/m^2. The soil mineralisable N in the pots of the current experiment were also below the optimal values of 10 – 15 g N/m^2 needed to provide forages with adequate soil N for high herbage DM yields and development (Sparling and Schipper 2002). This indicated that the soils were either N deficient, or that the available

N in the soil was not completely detected due to the process of mineralisation after the pots were deconstructed (Jarvis *et al.* 1996).

Soil mineralisable N in the pots after the final harvest was higher in the grasses (8.0 g/m^2) than the herbs (5.9 g/m^2) suggesting herbs have the ability to take up more N than grasses. This reflects the larger root systems in chicory and plantain that have the potential to take more N from the soil than other forages (Berendse 1982; Skinner *et al.* 2004). However, total N uptake and herbage N concentration in both plantain and chicory were not more than grasses suggesting that chicory and plantain may store more N in the roots and base of the plant rather than the leaf. As it was not possible to test the root N concentrations in the current experiment, more research is needed around root architecture and root N concentrations to confirm this theory.

From the viewpoint of reducing N leaching from the soil, the forage with lowest N uptake from the soil were Italian ryegrass (8.3 g N/m^2) and diploid perennial ryegrass (8.1 g N/m^2). This most likely reflects their low herbage DM yields and suggests they are not suited as forages to reduce N losses from the soil by higher N uptake in plants. However, in other related work by Moir *et al.* (2013), Italian ryegrass has the highest in N uptake across a comparison of 16 grass forages when measured over a winter period in a glasshouse. In addition, in previous chapters (Chapter 3, section 3.3.3), high herbage DM yield of Italian ryegrass in the winter/early spring period contributed to a higher N uptake in the herbage. Considering the risk of N leaching is greatest in the autumn and winter, when cooler temperatures reduce plant N uptake, and higher rainfall increases drainage of mineralisable N (Di and Cameron 2002c), the timing of current experiment may be a limitation to understanding N uptake in plants at times of year it is most important. This is because the current experiment did not include late autumn and winter seasons and was carried out in a glasshouse where temperatures were warmer than outside thus effecting the herbage DM yield and N uptake of the plant.

N fertiliser effect

Herbage N concentration increased when applications equal to or greater than 10 g N/m^2 were applied to the forages, although large differences in herbage N concentration were not evident until the highest N rate was applied. This result is consistent with previous chapters (Chapter 3, section 3.3.3) and indicates the difficulty of altering herbage N concentration in plants by altering N fertiliser regime. Herbage N concentration was highest in the 40 g N/m^2 N fertiliser rate (2.5 % N) which decreased plant N use efficiency. However, the herbage N concentrations are not high enough to be surplus to animal demands (2.8 % N) in the spring and summer (Pacheco and Waghorn 2008).

At the 0 g N/m^2 N fertiliser treatment, herbage N concentrations of perennial ryegrass (2% N), cocksfoot (2.2 % N) and Italian ryegrass (1.9% N) were considerably lower than the pot experiment carried out by Moir *et al.* (2013) at the same N fertiliser treatment (2.9 % N in perennial ryegrass, 3.1

% N in cocksfoot and 2.8 % N in Italian ryegrass). This result suggests the plants in the current trial at the 0 g N/m^2 were N deficient and also suggests the herbage N concentrations are too low to provide animals with sufficient protein for growth and milk production (Pacheco and Waghorn 2008). The reason for the large difference between this study and Moir *et* al (2013) is likely due to the higher mineralisable N in the soil in Moir *et al* (2013) (16.0 g N/m^2) compared to the current experiment (5.3 g N/m^2 prior to the experiment starting).

5.5 Conclusions and implications

The main implications drawn from this experiment are:

- Accumulated herbage DM yield was highest and N concentration lowest in prairie grass and plantain in the summer and autumn indicating it is the most practical to reducing nitrate leaching by providing animals with lower amounts of N in the diet per g DM, which reduces urinary N without impeding on farm production.

- Cocksfoot and prairie grass were the most responsive in DM to increasing soil N supply which proposes a lower rate of N fertiliser is needed to obtain similar herbage DM yields. This suggests they are more compatible to reduce N inputs into a farm system without reducing production of pasture.

- Low N fertiliser rates of below 17.5 g N/m^2 over 6 months can significantly impact on DM response rates due to a nutrient deficiency. Consequently, high N fertiliser rates of 40 g N/m^2 over 19 weeks N concentration of the forage increasing the risk of higher dietary N for animals which can lead to greater urinary N losses and therefore nitrate leaching.

Chapter 6

Effects of nitrogen fertiliser on herbage nitrogen solubility of alternative pasture forages in summer and autumn

6.1 Introduction

Nitrogen (N) in herbage can take different forms and these can influence how they are digested in the rumen and partitioned to product (Bach *et al.* 2005; Castillo *et al.* 2001; Lapierre *et al.* 2005; Pacheco and Waghorn 2008). For example, soluble forms of nitrogen (N) are at greater risk of being converted to ammonia rapidly in the rumen which, when in excess of energy source, causes large amounts of ammonia in the rumen, which is toxic to animals (Castillo *et al.* 2001; Pacheco and Waghorn 2008). As a result, the excess ammonia is removed from the rumen by absorption and transported to the liver. Here it is converted into urea and either recycled via saliva or more typically excreted in the urine (Castillo *et al.* 2001) causing higher N losses via the urine patch (Li *et al.* 2012). Consequently, N use efficiency (NUE) in the animal is lower (220 g N in milk/kg herbage N intake vs 290 g N in milk/kg herbage N intake (Kebreab *et al.* 2001)) because ammonia is lost as urea rather than converted into microbial protein for animal production. However, insoluble forms of N are slower to convert into ammonia and may escape rumen degradation altogether (Bach *et al.* 2005; Lapierre *et al.* 2005; Pacheco and Waghorn 2008), which may be more desirable to reduce N losses. This is because lower amounts of ammonia in the rumen equates to lower conversion of ammonia to urea in the liver, provided energy sources are sufficient for microbial protein synthesis. As a result, NUE is much higher because less ammonia is lost as urea and more is converted into microbial protein for animal production. With this in mind, when investigating alternative pasture forages to reduce N losses, some may have similar crude protein (CP) concentration, but are different in N solubility and therefore NUE (Bryant et al. 2012).

Plant soluble components in perennial ryegrass have been shown to be altered by different management strategies such as fertiliser (Goswami and Willcox 1969) and timing of grazing (Bryant *et al.* 2012; Hoekstra *et al.* 2008; Loaiza *et al.* 2016; Sanderson and Wedin 1989). For example, in Goswami and Willcox (1969), an increase in N fertiliser was shown to increase plant nitrate from 2.6 to 9.4 % of total N, and increase free amino acids (AA) in the plant from 10.4 to 15.0 % of total N. Furthermore, Bryant et al. (2012) and Loaiza *et al.* (2016) showed soluble N of perennial ryegrass increased as regrowth interval increased from 1 to 4 weeks (50% to 57% in Bryant et al. (2012) and 339 g/kg CP to 408 g/kg CP in Loaiza *et al.* (2016)). In addition, in legume forages, Kirchhof *et al.* (2010) who found soluble N fractions (A and B1) were lowest in the birdsfoot trefoil (319 g/kg CP) and highest in the

lucerne and white clover (529 and 525 g/kg CP, respectively) However, there is little comparative information on N solubility of herbs. In addition, higher water soluble carbohydrates (WSC) in perennial ryegrass diets have been suggested to improve NUE through better synchrony of energy and N sources in the rumen (Holmes et al. 2007b; Miller et al. 2001; Pichard and Van Soest 1977). However, few studies on alternative pasture forages have been conducted (Brown and Pitman 1991; Bryant et al. 2012; Hoekstra et al. 2008; Kirchhof et al. 2010).

Therefore, the objective of this experiment was to quantify the effect on N fertiliser rate on chemical composition and N solubility in plantain, chicory, lucerne, white clover, diploid perennial ryegrass and cocksfoot at optimum defoliation time in the summer and autumn.

6.2 Materials and methods

6.2.1 Site and experimental design

This experiment was conducted using the herbage collected in Chapter 3. The experimental site was at LURDF, Canterbury, New Zealand (43°64'S, 172°46'E) under irrigation on a free-draining Templeton fine sandy loam (Immature Pallic soil) (Hewitt 2010). Of the 12 forages sown, 6 were used in this experiment. The experiment was a split-split plot design with the 6 pasture forages grown at two different N fertiliser rates and over two seasons with three blocks. Samples were taken from the site 22 and 24 months after sowing in the 2015/16 growing season. The six pasture forages (Table 6.1) were the main plot treatments, the two fertiliser rates were the split-plot treatments and the two seasons (summer and autumn) were the split-split plot treatments. These seasons were chosen because of their high (autumn) and low (summer) risk of nitrate leaching occurring. The N fertiliser rates were split into two categories. These were medium and high N fertiliser. The medium N fertiliser rate was 180 kg N/ha/year for grasses and herbs and 156 kg N/ha/year for legumes. The high N fertiliser rate was 450 kg N/ha/year for grasses and herbs and 389 kg N/ha/year for legumes. The forages were selected for this experiment because they were high in herbage DM yield (e.g. plantain, white clover, lucerne), low in N concentration (diploid perennial ryegrass, plantain) or responded differently than expected in Chapters 3, 4 and 5. For example cocksfoot and chicory were higher in N concentration than expected.

Table 6.1 Forages sown and their functional group, scientific name, cultivar and sowing rate.

Pasture	Functional group	Scientific name	Cultivar	Sowing rate (kg/ha)
Diploid PRG – late season flowering	Grass	*Lolium perenne*	One-50 (AR37*)	20
Cocksfoot	Grass	*Dactylis glomerata*	Savvy	8
Chicory	Herb	*Cichorium intybus*	Choice	8
Plantain	Herb	*Plantago lanceolata*	Tonic	10
White clover	Legume	*Trifolium repens*	Kopu 2	5
Lucerne	Legume	*Medicago sativa*	Torlesse (Superstrike**)	14

Diploid PRG, diploid perennial ryegrass.
* Endophyte
** Seed coating

More details on the establishment, management and irrigation can be found in Chapter 3 (section 3.2.1). However, in brief, the experiment was established in March 2014, following cultivation. Herbage was controlled using mower harvests starting in spring 2014 which avoided trampling and nutrient recycling by livestock. Plots were mowed to a 4 cm height to mimic grazing from cows. In grasses and herbs harvests were carried out in spring, summer and autumn every 32, 26 and 30 days, respectively, to allow sufficient regrowth (Donaghy and Fulkerson 1998; Lee *et al.* 2015). In legumes,

harvests were carried out in spring, summer and autumn every 41, 35 and 41 days, respectively, to allow sufficient regrowth (Moot *et al.* 2003). Nitrogen fertiliser was applied following each harvest as calcium ammonium nitrate (27 : 0 : 0 : 0; N : P : K : S), with the total annual N application rate split throughout the year. This is shown in Table 6.2. Climate data was collected from Broadfields Meteorological Station, 1 km from the experimental area. Annual irrigation was 550 mm, applied between October and March.

Table 6.2 Single N fertiliser rates to plots for three N fertiliser treatments in spring and autumn 2015.

	Annual N application		Single N application	
N fertiliser treatment	**Grasses and herbs**	**Legumes**	**Grasses and herbs**	**Legumes**
	--------- kg N/ha/yr ---------		------ kg N/ha/application -------	
Medium	180	156	20	22
High	450	389	50	56

6.2.2 Herbage measurements

For the summer harvest, grass and herb samples were collected from the experimental site on 1 February 2016 and legume samples were collected on 23 February 2016. For the autumn harvest, grass, herb and legume samples were collected from the experimental site on 30 March 2016. At each harvest, two samples of approximately 300 g of fresh herbage was collected from each plot using hand shears, with an attachment set to 4 cm height. The first sample was used to determine chemical composition and the second sample was used to quantify botanical composition. Harvesting occurred between 10:00 am and 12:00 pm avoiding previously harvested areas. Harvested samples were kept in the shade until all the plots had been harvested. They were then transported to the laboratory. The chemical composition sample was stored at -18 ^{o}C. Once frozen, the herbage was freeze-dried and ground through a 1 mm sieve with a M200 rotor mill (Retsch Inc., Newtown, Pennsylvania, USA) ready for N fractionation analysis. The botanical composition sample was stored in the fridge at 4^{o}C and then hand sorted into leaf, stem and dead material. It was then oven-dried for 48 hours at 60^{o}C, weighed and, plant morphological percentage determined on a DM basis.

6.2.3 Herbage total N

All analysis was conducted at the Lincoln University analytical laboratory. Herbage total N concentration was determined using combustion under oxygen (Elemental Analyser vario MAX CN, Analysensysteme GmbH. Hanau, Germany). However, Licitra *et al.* (1996) recommended herbage total N be measured using the Kjeldahl method and Bryant *et al* (2012) used near-infrared spectrophotometry (NIRS, NIRSystems 5000, Foss, Maryland, USA). Therefore, a small number of selected samples from each forage (n = 32) were also analysed for herbage total N using Kjeldahl (Bremner 1965) and NIRS techniques to compare between the different analytical methods. NIRS

results were based on calibrations derived on the experimental herbages in Chapter 3, (section 3.3.3). Any samples outside the calibration spectrum, were analysed by wet chemistry using the methods described in Chapter 3, (section 3.2.3). The NIRS method determines herbage crude protein (CP) concentration and therefore, to calculate herbage N concentration, CP was divided by 6.25. Results were plotted against each other to identify relationships between the different methods and check it was acceptable to use the Elementa method to measure herbage N concentration.

The relationship between combustion under oxygen (Elemental Analyser vario MAX CN, Analysensysteme GmbH. Hanau, Germany) and NIRS (Figure 6.1 (a)) (Model FOSS NIRSystems 500, Maryland, USA) was strong (R^2 =0.99) with Elementa results (3.5 % total N) only 0.13 % higher in N concentration compared to NIRS (3.5 % total N) when comparing between the two methods.

The relationship between combustion under oxygen (Elemental Analyser vario MAX CN, Analysensysteme GmbH. Hanau, Germany) and Kjeldahl technique (Figure 6.1 (b)) was shown to be also strong (R^2 = 0.99) with 0.37 % higher in N concentration using Elementa than Kjeldahl (2.9 % total N).

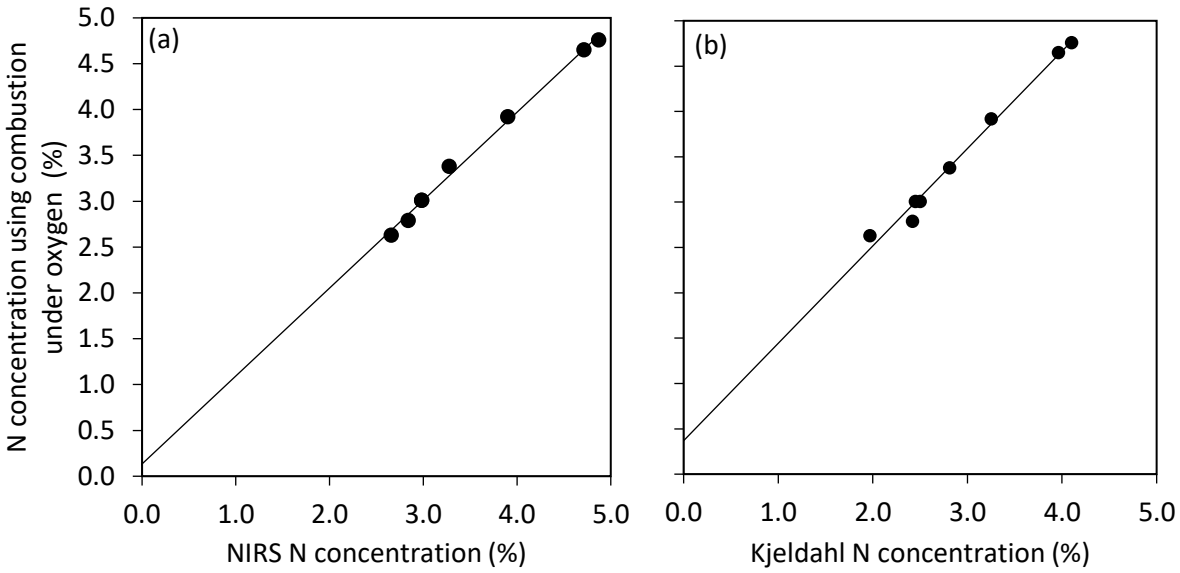

Figure 6.1 Linear relationship between (a) N concentration measured by combustion under oxygen (Elemental Analyser vario MAX CN, Analysensysteme GmbH. Hanau, Germany) and NIRS (Model FOSS NIRSystems 500, Maryland, USA) and (b) combustion under oxygen (Elemental Analyser vario MAX CN, Analysensysteme GmbH. Hanau, Germany) and Kjeldahl technique (Bremner 1965). Regression of graph (a) is: y = 0.96x + 0.13 (R^2 = 0.99) and graph (b) is: y = 1.1x + 0.37 (R^2 = 0.99).

6.2.4 **N fractionation**

Nitrogen solubility of the forages were measured as N fractions; fraction A (NPN), fraction B1 (soluble N), fraction B2 (insoluble N, but soluble in neutral detergent fibre (NDF)) and fraction B3 and C (neutral detergent insoluble N (NDIN)), using the freeze dried herbage samples taken from the field. The N

fractions were determined using the procedures of Licitra *et al.* (1996) and duplicates of each fraction (fraction A, B1, B2 and B3+C) for every sample were taken. Results with a variance greater than 5 % between duplicates were repeated. Due to unexpectedly large variance in a large proportion of the samples, there was insufficient plant material to complete the acid detergent insoluble N (ADFN), (C fraction). Instead NDIN was used to represent both rumen undegradable protein fractions, B3 and C. Blank samples were also included during the washing and filtering process to account for any N in the filter paper and reagents. The filter paper was then analysed using combustion under oxygen (Elemental Analyser vario MAX CN, Analysensysteme GmbH. Hanau, Germany). Sample processes are explained in more detail below.

Non protein N analysis (A fraction)

For NPN, 0.5 g (DM) of ground sample was weighed into a 125 ml Erlenmeyer flask. A total of 50 ml of cold, distilled water was added and left to stand (Plate 6.1 (a)). Thin plastic wrap was used to seal the top of the flask in order to avoid dust contamination. After 30 minutes, 10 ml of 10 % trichloroacetic acid (kept refrigerated) was added to the sample and left to stand for a further 30 minutes (Plate 6.1 (b)). The sample was then filtered using # 541 paper, (which had been previously weighed), by gravity and washed twice with the same solution (Plate 6.1 (c)). When filtration took > 1 hour, filters were covered to avoid dust contamination. The washed sample with filter paper was placed in a 100 ^{o}C oven for 12 hours to dry. The dried sample was weighed to obtain digested weight of the sample, with the weight of the filter paper taken off the total sample weight. A subsample was scraped off the filter paper and analysed to determine the residue N using combustion under oxygen (Elemental Analyser vario MAX CN, Analysensysteme GmbH. Hanau, Germany). NPN was calculated by subtracting residue N from total N.

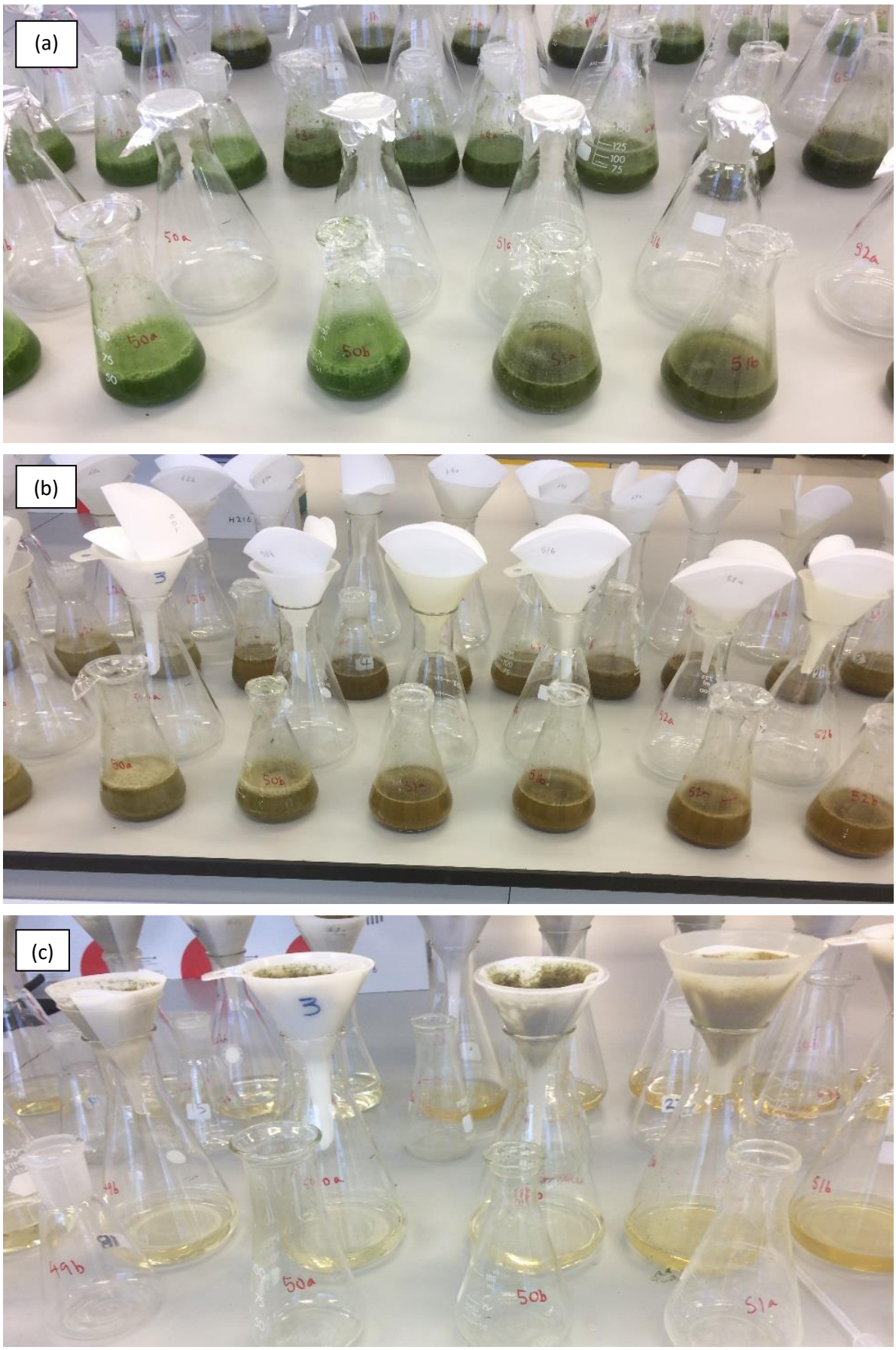

Plate 6.1 (a) 0.5 g DM forage sample in 125 ml Erlenmeyer flask: with 50 ml of cold distilled water, (b) with 10 ml of 10 % trichloroacetic acid and (c) filtered using #541 paper and washed twice with the same solution.

Insoluble protein

For insoluble protein, 0.5 g (DM) of ground sample was weighed into a 125 ml Erlenmeyer flask. A total of 50 ml of borate-phosphate buffer and 1 ml of sodium azide solution (freshly prepared) was added and left to stand at room temperature (Plate 6.2 (a)). Thin plastic wrap was used to seal the top of the flask to avoid dust contamination. After 3 hours, the sample was filtered using 12.5 mm Whatman # 541 paper, (which had been previously weighed), by gravity and washed with 250 ml cold distilled water (Plate 6.2 (b)). When filtration took > 1-hour, filters were covered in order to avoid dust contamination. The washed sample, including filter paper, was placed in a 100 ^{o}C oven for 12 hours. The dried sample was weighed to obtain digested weight of the sample, with the weight of the filter paper taken off the total sample weight. A subsample was scraped off the filter paper and analysed to determine the residue N using combustion under oxygen (Elemental Analyser vario MAX CN, Analysensysteme GmbH. Hanau, Germany). The insoluble protein fraction, was calculated by subtracting residue N from total N.

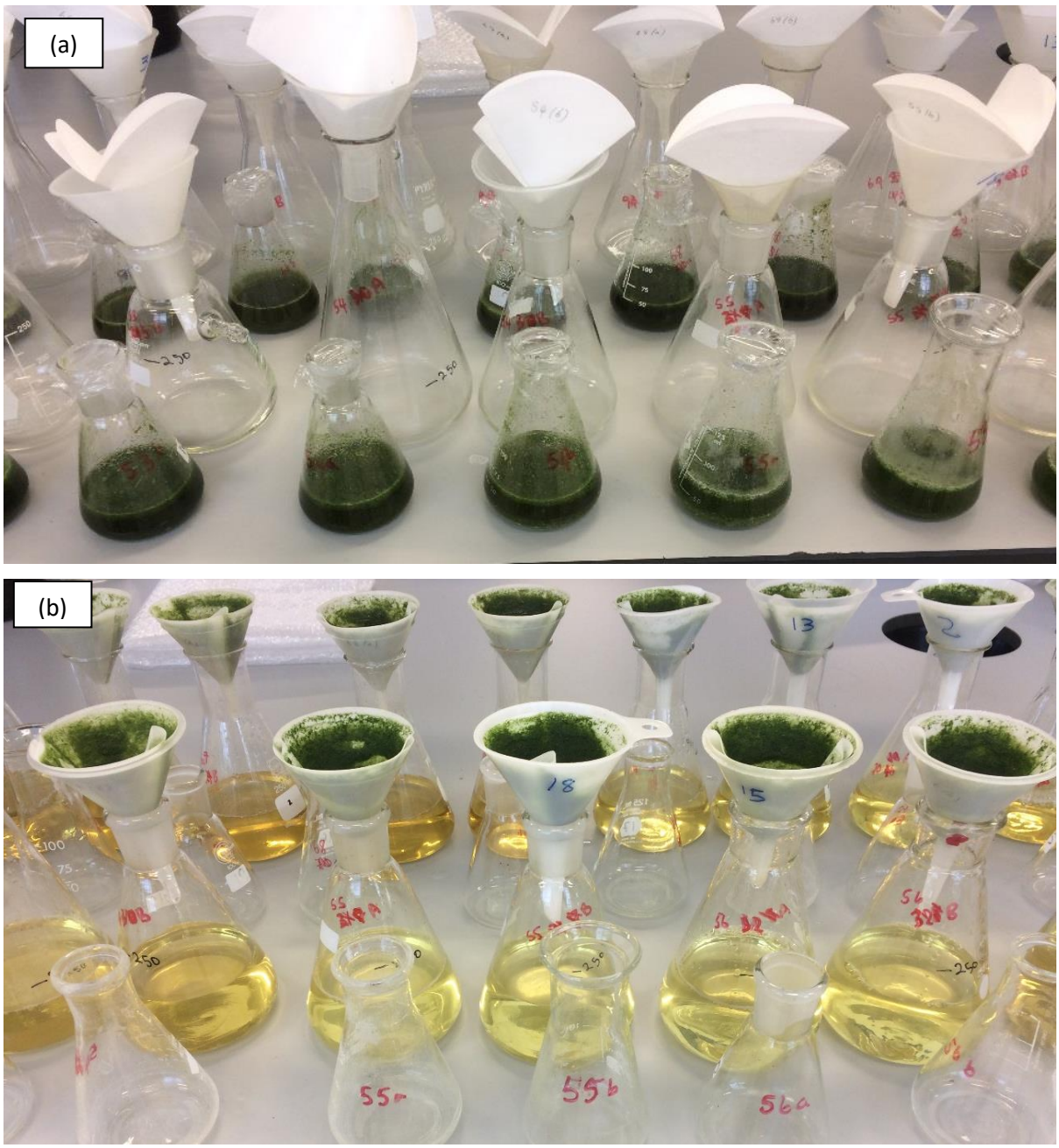

Plate 6.2 0.5 g DM forage sample in 125 ml Erlenmeyer flask: with 50 ml of borate-phophate buffer and 1 ml of sodium azide solution (freshly prepared) added and left to stand at room temperature (a) and filtered using 12.5 mm Whatman #541 paper by gravity and washed with 250 ml cold distilled water (b).

Soluble protein

Soluble protein was calculated using the difference between insoluble protein and total N:

Soluble protein = total N – insoluble N

True soluble protein (B1 fraction)

True soluble protein was calculated by subtracting the NPN fraction from the soluble protein:

True soluble protein (B1 fraction) = soluble protein - NPN

NDIN analysis (B3 and C fractions)

For NDIN, 1 g (DM) of ground sample was weighed into a 600 ml beaker and 50 ml of NDF solution added. The beaker was then placed onto a heated plate and a condenser fitted on top (Plate 6.3). The sample was brought to the boil and refluxed for 1 hour. Any samples that foamed vigorously at the start of heating were rinsed on the sides of the beaker with more NDF solution. The sample was filtered with mild suction on 12.5 mm Whatman #541 paper, (which had been previously weighed), and washed with hot distilled water until detergent free. The washed sample with filter paper was placed in a 100 ^{o}C oven for 12 hours. The dried sample was weighed to obtain digested weight of the sample with the weight of the filter paper taken off the total sample weight. A subsample was scraped off the filter paper and analysed to determine the residue N using combustion under oxygen (Elemental Analyser vario MAX CN, Analysensysteme GmbH. Hanau, Germany).

Plate 6.3 diploid perennial ryegrass sample with NDF solution on heating plates which were boiled for 1 hour

Insoluble protein, but soluble in NDF (B2 Fraction)

Insoluble protein, but soluble in NDF, was calculated by the difference between the insoluble protein and NDIN using the following equation:

Insoluble protein, but soluble in NDF (B2 Fraction) = insoluble protein – NDIN

6.2.5 WSC and CP concentration

The WSC and CP concentrations of the herbage were calculated using NIRS (NIRSystems 5000, Foss, Maryland, USA) techniques, based on calibrations derived on the experimental herbages in Chapter 3, (section 3.3.3). Any samples outside the calibration spectrum, were analysed by wet chemistry using the methods described in Chapter 3, (section 3.2.3).

6.2.6 Statisical analysis

The effects of N fertiliser treatment (N), forage (F) and season (S) and their interactions on individual N fractions and the WSC:CP ratio were compared by ANOVA (Genstat Version 16, VSN International Ltd) using a split – split - plot model. In the statistical model, forage was the main plot factor (n=6), N fertiliser rate was the split plot factor (n=2) and season was the split-split plot factor (n=2). Block was the residual error (n=3).

6.3 Results

6.3.1 Meteorological data

During the experiment, total rainfall, air temperature and soil temperatures in summer and autumn 2016 were higher than the long-term average. More detailed conditions are shown in Chapter 3 (section 3.3.1).

6.3.2 Plant morphology of harvested herbage

The statistical effects of treatments on plant morphology of the forages, as determined by ANOVA, are presented in the Appendix Table C. 1.

Morphology of the forages at each N fertiliser rate are shown in Figure 6.2. An interaction (P<0.001) between N fertiliser and forage showed that as N fertiliser increased from medium to high, dead matter decreased in cocksfoot and diploid perennial ryegrass, and stem decreased in lucerne (Figure 6.2). However, herbage samples for chicory, plantain and white clover were made up entirely (100 %) of leaf.

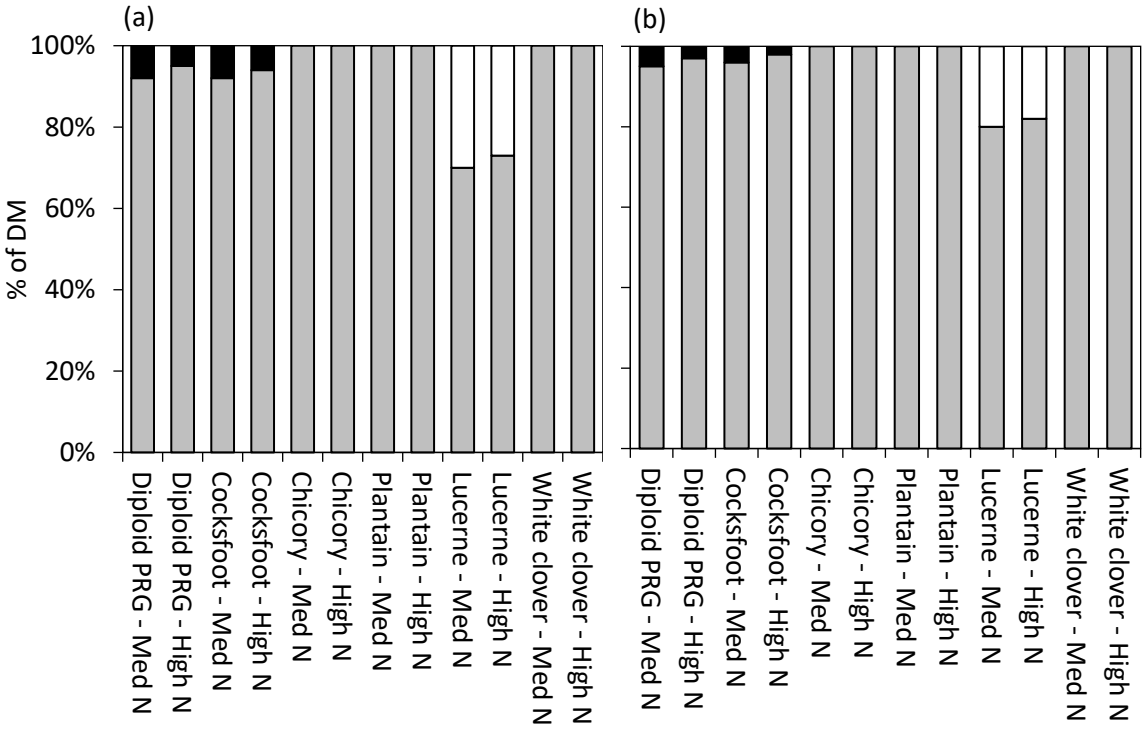

Figure 6.2 Percentage of leaf (grey), stem (white) and dead matter (black) in six forage species over two N fertiliser treatments, medium (med) and high in summer (a) and autumn (b). Diploid perennial ryegrass, diploid perennial ryegrass.

6.3.3 Total N concentration

The statistical effects of treatments on herbage N concentration are presented in the Appendix Table C. 2.

In general, N concentration as a % of DM was lowest (P<0.001) in diploid perennial ryegrass and plantain (3.0 % N – 3.1 % N in DM) and highest in white clover (4.8 % N in DM). An interaction between forage and N fertiliser rate showed total N of both grasses and herbs was greater in the high N fertiliser rate than the medium N fertiliser rate (P<0.001). However, no response occurred in the legumes (Figure *6.3*). In addition, seasonal effects showed total herbage N concentration was lower in summer than autumn (3.1 % N vs 3.8 % N, P<0.001).

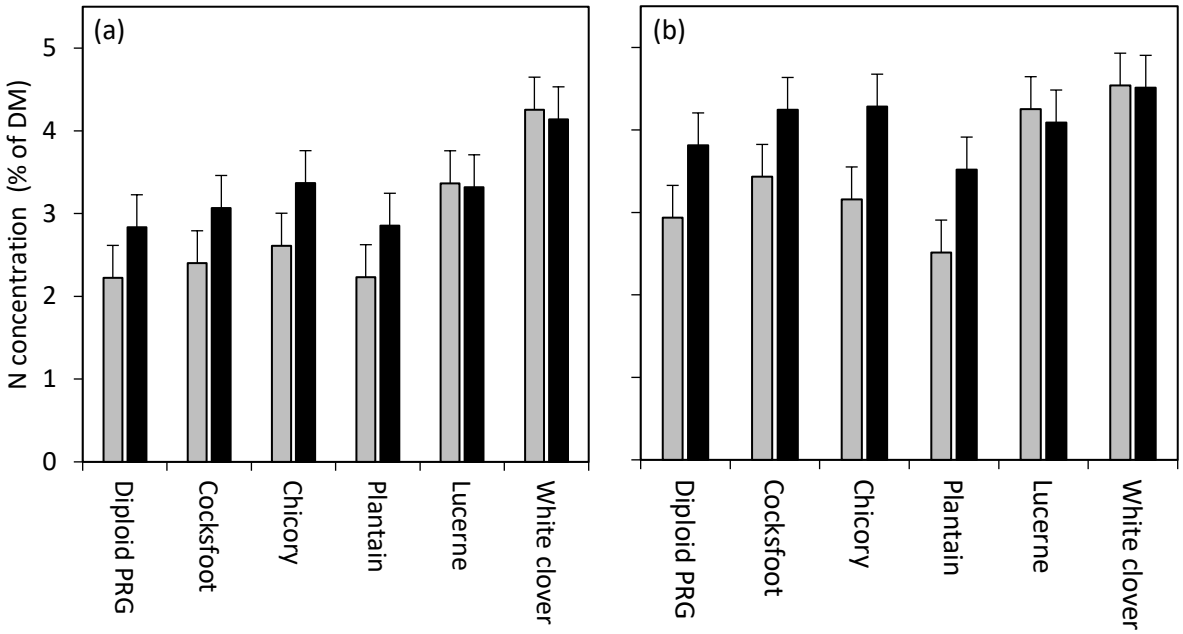

Figure 6.3 Total N concentration (% of DM) of six forages under two fertiliser regimes of medium (grey) and high (black) in summer (a) and autumn (b). Error bars are species x N rate LSD values when comparing means with the same level of forage. Diploid perennial ryegrass, diploid perennial ryegrass.

6.3.4 N fractions

Fractionation of N as a proportion of DM and total N are presented in Figure 6.4 and Figure 6.5 and Appendix Table C. 2 and Table C. 3 presents statistical effects of treatments.

In herbs and legumes, the largest fraction was B2, and in grasses was NDIN (P<0.001). Generally, chicory and legumes had the greatest proportion and quantity of soluble N (NPN and B1), followed by grasses and plantain which contained the lowest solubility (B2 and NDIN).

Fraction A – soluble non-protein N (NPN)

Non protein N as a % of DM was greater in the legumes (0.8 % N in DM) than grasses (0.4 % N in DM) or herbs (0.3 % N in DM, P<0.001, Figure 6.4, respectively). An interaction between forage and N fertiliser showed at medium N fertiliser, NPN in grasses and herbs were lower than at high N fertiliser. However, there was no effect shown in the legume forages. A further interaction with season showed the increasing effect of N fertiliser on NPN was more pronounced in autumn (increase of 0.13 % N) than summer (increase of 0.32 % N) (Figure 6.4).

Fraction B1 – soluble true protein N

On a DM basis (% N in DM), chicory and white clover had a greater fraction B1 (1.4 % N – 1.5 % N in DM) than plantain and diploid perennial ryegrass (0.8 % N in DM). An interaction (P<0.01) between forage and N fertiliser, showed that as N fertiliser increased in the herbs, fraction B1 was lower at the medium N fertiliser (1.3 % N in chicory and 0.6 % N in plantain) than the high N fertiliser (1.5 % N in chicory and 0.9 % N in plantain). However, no effect of N fertiliser occurred in legumes or grasses (Figure 6.4). Moreover, seasonal differences (P<0.001) showed fraction B1 were lower in summer compared to autumn (0.9 % N vs 1.2 % N in DM, respectively, Figure 6.4).

When expressed as a proportion of total N (mg/g N), there was no interaction between forage and N fertiliser. However, differences in the main effects were found (Figure 6.5). Fraction B1 was lower (P<0.001) in grasses and plantain (230 - 263 mg per g of N (mg/g N), averaged across N fertiliser rates, and highest in chicory (395 mg/g N). Fraction B1 was lowest at the high N fertiliser rate (275 vs 293 mg /g N, P<0.01) and higher in autumn than spring (304 vs 264 mg/g N, P<0.001).

Fraction B2 – insoluble, degradable N

On a DM basis (% N in DM), N concentration of fraction B2 was greatest in white clover (2.4 % N in DM) and lowest in diploid perennial ryegrass (0.8 % N in DM). An interaction between N fertiliser and forage (P<0.001) showed grasses and herbs had greater fraction B2 at high N fertiliser rates than medium N fertiliser rates. However, no effect of fertiliser was observed in legumes (Figure 6.4). In addition, a seasonal effect showed that fraction B2 was greater (P<0.001) in autumn (1.7 % N in DM) than summer (1.4 % N in DM) (Figure 6.4).

When expressed as a proportion of total N (mg/g N), differences between forages showed fraction B2 was greatest in plantain (570 mg/g N) and lowest in diploid perennial ryegrass (245 mg/g N). However, there were no differences between N fertiliser and season (Figure 6.5).

Fraction B3 and C - neutral detergent insoluble N (NDIN)

On a DM basis (% N in DM), NDIN ranged from 0.1 % N – 1.4 % N among the forages (P<0.001) with cocksfoot containing the greatest proportion (1.3 % N in DM) and white clover and chicory containing

the least (0.2 % N in DM) (Figure 6.4). However, NDIN did not differ between the two seasons and was not affected by N fertiliser treatment.

When expressed as a proportion of total N (mg/g N), an interaction between forage and N fertiliser rate showed diploid perennial ryegrass, cocksfoot and plantain were lower in NDIN at the high N fertiliser rate compared to the medium N fertiliser rate. However, NDIN did not differ between N fertiliser in the lucerne, white clover or chicory forages. In addition, an interaction between season and forage showed NDIN in grasses was higher in summer than autumn (428 vs 333 mg/g N, respectively). However, NDIN in herbs and legumes was not affected by season.

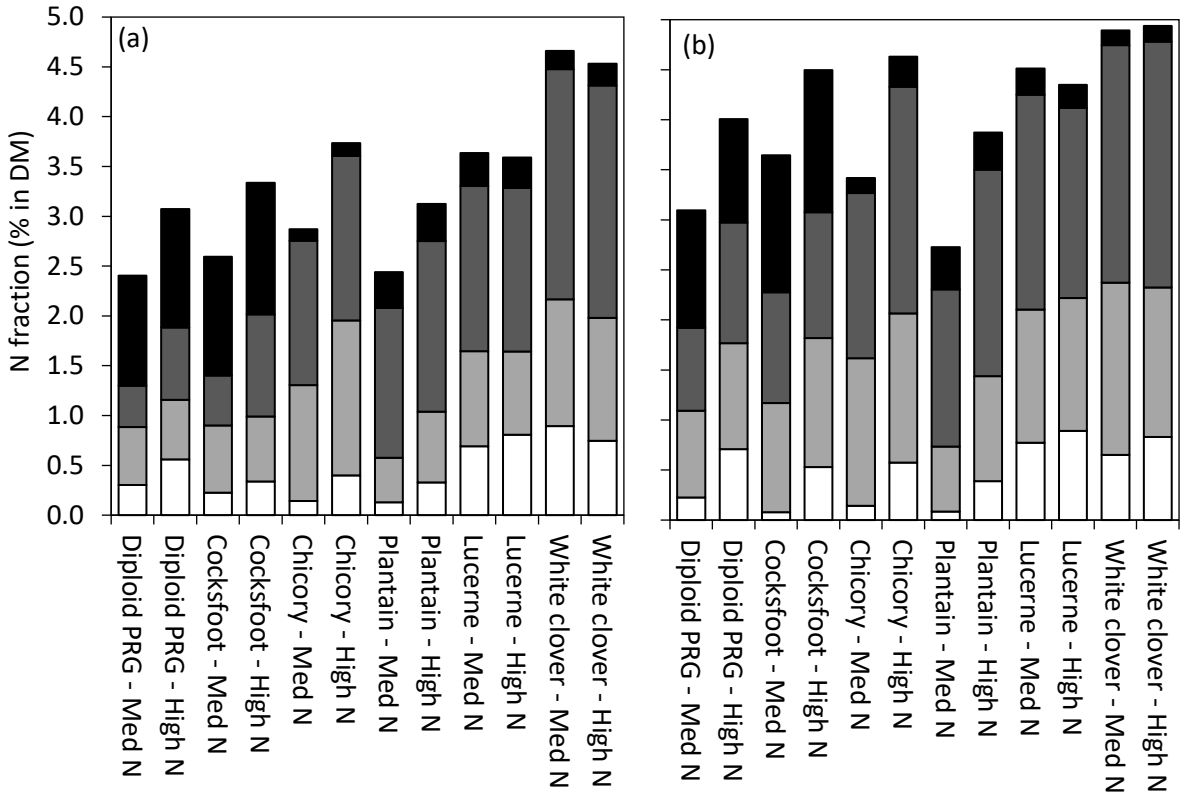

Figure 6.4 Total herbage N concentration as a % of DM, split into NPN (white), fraction B1 (light grey), fraction B2 (dark grey) and NDIN (black) of six forages under two N fertiliser regimes (medium and high) in summer (a) and autumn (b). Diploid PRG, diploid perennial ryegrass.

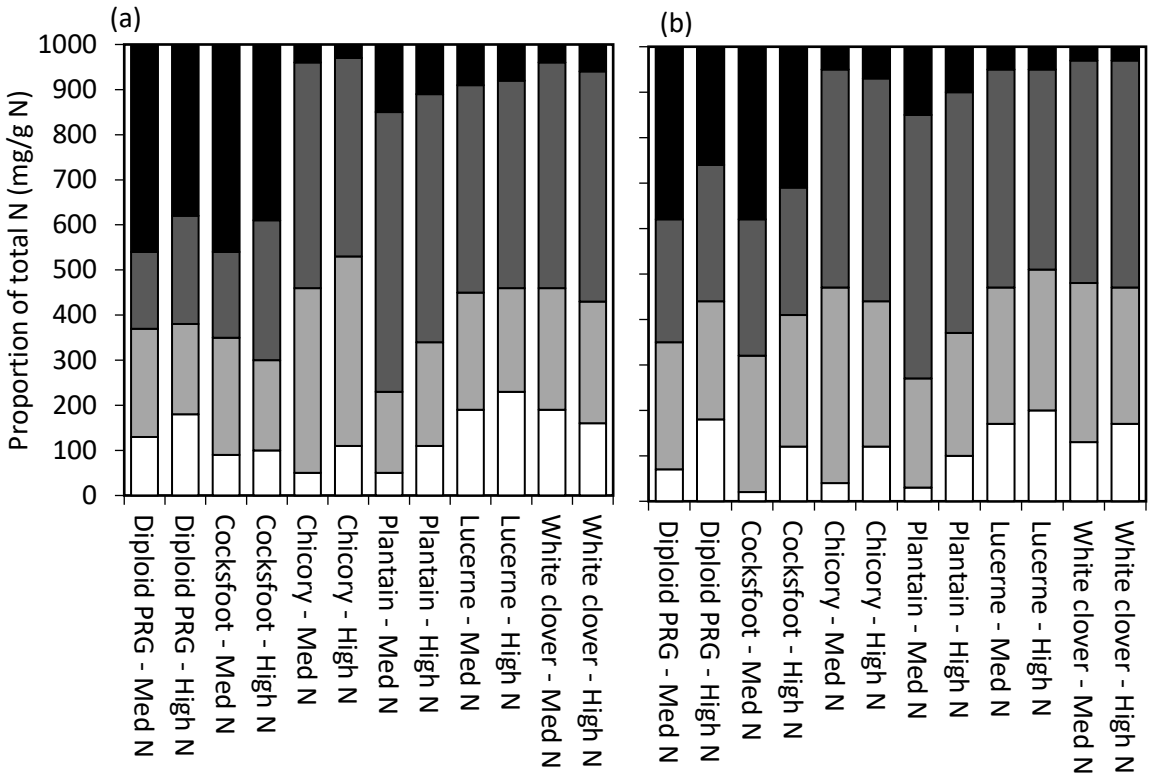

Figure 6.5 Nitrogen fractions when expressed as a proportion of total N, split into NPN (white), fraction B1 (light grey), fraction B2 (dark grey) and NDIN (black) of six forages under two N fertiliser regimes (medium and high) in summer (a) and autumn (b). Diploid PRG, diploid perennial ryegrass.

6.3.5 WSC, CP and WSC:CP ratio

The statistical effects of treatments on the herbage N concentrations and crude protein (CP) are presented in Table 6.3.

An interaction in herbage WSC concentrations between season and forage showed diploid perennial ryegrass and herbs were higher in the WSC in the summer compared to autumn. However, there was no seasonal difference in the legumes or cocksfoot. N fertiliser rate did not affect the WSC concentrations in any of the forages, in either of the seasons. An interaction in CP concentrations between N fertiliser rate and forage showed diploid perennial ryegrass, cocksfoot and plantain had higher CP concentrations at the high N fertiliser rate than medium N fertiliser rate. However, N treatment did not affect the CP concentrations in chicory or legume forages.

Table 6.3 WSC and CP of six forages under two N fertiliser regimes (medium and high) in summer and autumn.

Season	Species	N Rate	WSC % of DM	CP % of DM
Summer	Diploid perennial ryegrass	Medium	20.9	15.1
Summer	Diploid perennial ryegrass	High	18.6	18.2
Summer	Cocksfoot	Medium	9.4	15.7
Summer	Cocksfoot	High	9.1	19.0
Summer	Chicory	Medium	19.7	19.3
Summer	Chicory	High	18.6	22.9
Summer	Plantain	Medium	22.9	16.9
Summer	Plantain	High	21.2	20.0
Summer	Lucerne	Medium	8.7	24.3
Summer	Lucerne	High	9.6	23.9
Summer	White clover	Medium	14.2	28.5
Summer	White clover	High	15.4	27.5
Autumn	Diploid perennial ryegrass	Medium	15.3	19.7
Autumn	Diploid perennial ryegrass	High	13.1	23.8
Autumn	Cocksfoot	Medium	7.6	22.7
Autumn	Cocksfoot	High	8.8	26.6
Autumn	Chicory	Medium	14.7	24.7
Autumn	Chicory	High	16.3	24.6
Autumn	Plantain	Medium	15.7	19.5
Autumn	Plantain	High	18.0	24.6
Autumn	Lucerne	Medium	8.7	28.8
Autumn	Lucerne	High	10.6	27.3
Autumn	White clover	Medium	16.5	30.6
Autumn	White clover	High	15.5	30.8
	d.f		F Pr.	F Pr.
Forage (F)	5		***	***
N fertiliser (N)	1		NS	***
F x N	5		NS	**
Season	1		**	***
F x S	5		**	NS
N x S	1		NS	NS
F x N x S	5		NS	NS

NS, not significant; d.f, degrees of freedom; F pr., significance value.
* P≤0.05
** P<0.01
***P<0.001

The statistical effects of treatments on the WSC:CP ratio are presented in Figure 6.6 and the Appendix Table C. 2.

The ratio of WSC to CP was analysed as a proxy indicating the degree of synchrony between energy and protein supply in the rumen (Edwards *et al*. 2007). An interaction between season and forage showed grasses and herbs were higher in the WSC:CP ratio in the summer compared to autumn. However, there was no seasonal difference in the legumes. Likewise, an interaction between N fertiliser rate and forage showed diploid perennial ryegrass and plantain had lower WSC:CP ratios at

high N fertiliser rates than medium N fertiliser rates. However, N treatment did not affect the WSC:CP ratio in cocksfoot, chicory or legume forages.

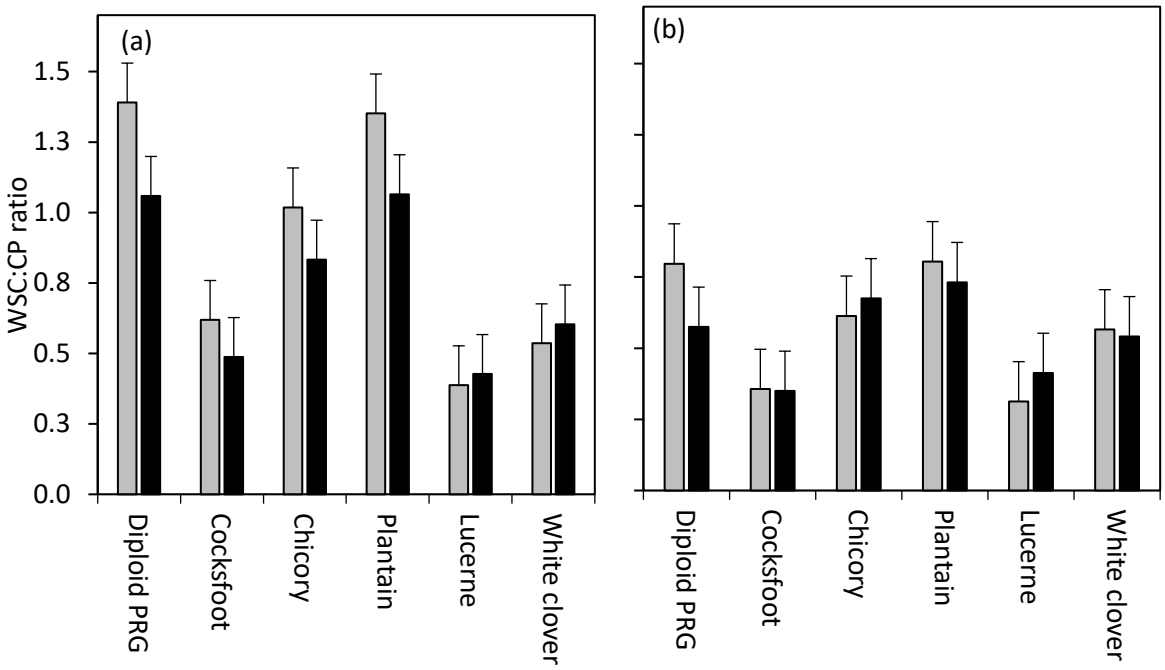

Figure 6.6 Average WSC:CP ratio of six forages under two fertiliser regimes of medium (grey) and high (black) in summer (a) and autumn (b). Error bars are species x N rate LSD values when comparing means with the same level of forage. Diploid PRG, diploid perennial ryegrass.

6.4 Discussion

6.4.1 N fractions

Forage effect

Supplying animals with a diet that has a high N solubility (NPN and B1) is less desirable to reduce N losses because the readily available N is rapidly absorbed via the rumen, converted into urea in the liver and then excreted into the urine (Pacheco and Waghorn 2008). The current experiment showed soluble N (NPN and B1) was lowest in plantain (303 mg/g N), intermediate in grasses (365 mg/g N) and highest in legumes and chicory (460 mg/g N – 475 mg/g N). Previous studies between grass and legumes, have made similar conclusions; Brown and Pitman (1991) found legumes were higher in highly soluble N fraction compared to grasses and Kirchhof *et al.* (2010) showed similar proportions of soluble N to the current experiment in the spring (512 mg/g N vs 466 mg/g N). However, previous reports of soluble N in perennial ryegrass differ. Bryant *et al* (2012) and Hoekstra *et al* (2008), showed soluble N in perennial ryegrass was between 410 mg/g N and 596 mg/g N which was significantly greater than the current experiment. The reasons for such a large difference may be due to differences in plant available N in the soil, which could lead to an N deficiency in the plants. Bryant *et al* (2012) grazed the pasture prior to harvesting, and therefore N in the form of urea from urine and faeces likely increased the amount of mineralisable N in the soil. In contrast, the current experiment was carried out using a cut and carry method with herbage removed.

Although there is limited comparative research available for the herb forages, an *in vitro* experiment carried out by Navarrete *et al.* (2016) showed ammonia production in the rumen of animals fed plantain was significantly lower than animals fed chicory. It was suggested this was because of higher concentrations of acteoside in plantain which provides microbes with higher fermentable carbohydrates such as non-specific glycosidase as an energy source (Getachew *et al.* 2002). This may increase energy availability and indicates more ammonia is incorporated into microbial protein improving the efficiency of N in the rumen and decreasing the amount lost as urea in the liver (Raab *et al.* 1983). In addition, Box *et al.* (2017) found of the secondary plant compounds measured, acteoside had the highest concentration in plantain, which coincided with lower urine N outputs. However, the increase in rumen ammonia production could also be due to lower levels of insoluble N in the plantain as found in the current experiment. As acteoside was not measured in the current experiment, further work should be carried out to confirm this suggestion.

Diets with a higher proportion of insoluble protein, but soluble in NDF (fraction B2) are more desirable to reduce N losses (Bach *et al.* 2005; Lapierre *et al.* 2005; Pacheco and Waghorn 2008). This is because the N is slower to degrade in the rumen and therefore more available for the microbes to process into microbial protein for absorption. In addition, plant N bound in the cellulose and lignin, otherwise

known as NDIN (fractions B3 and C), is assumed to be indigestible by the animal. Instead it is excreted in the faeces in a more stable form of N that is less likely to cause N leaching losses (Stout *et al.* 1997). Results from the current experiment found insoluble N (B2 and NDIN) was lowest in the legumes and chicory (525 mg/g N - 540 mg/g N), intermediate in grasses (638 mg/g N) and highest in plantain (700 mg/g N). Previous research by Kirchhof *et al.* (2010) showed similar results with 489 mg/g of N insoluble N in white clover and lucerne. The slightly lower values observed in this study was possibly because of an earlier harvest which resulted in lower cellulose and lignin in the stem. Comparable results by Hoekstra *et al* (2008) showed insoluble N in perennial ryegrass was 592 mg/g N. However, the partitioning of N between fraction B2 and NDIN was different in the current study than Hoekstra *et al* (2008). Hoekstra *et al* (2008) showed significantly lower NDIN (134 mg/g N) than the current experiment (380 mg/g N). In addition, Sanderson and Wedin (1989) also showed lower NDIN concentrations of 133 mg/g N in bromegrass and 214.5 mg/g N in timothy. Similar results were also found in Bryant *et al.* (2012) who showed NDIN values were considerably lower (92 mg/g N) than the current experiment results at the third leaf stage. The reason for a lower NDIN in previous studies compared to this study is unclear, however may be related to the lower proportion of dead material in the grass forages, which contains N bound in cellulose and lignin. As Bryant *et al.* (2012) harvested their herbage in spring to a height where little dead material was included, thus NDIN fraction was relatively low. However, the current trial was harvested in the summer and autumn when the quality of the forages may have been lower. This increased the NDF of the forage and therefore affected the NDIN concentration. This is demonstrated in the botanical composition of the forages which made up to 8 % dead matter in autumn, and 5 % dead matter in the spring. The NDIN concentrations in the lucerne was more comparable with previous experiments. For example, Sanderson and Wedin (1989) showed NDIN in lucerne was 61 mg/g N which is similar to the NDIN of lucerne in the current study (70 mg/g N, averaged over the N fertiliser rates and seasons). The high level of insoluble N in plantain could be explained by the presence of acteoside in the plant, which was mentioned in the previous paragraph and in Navarrete *et al.* (2016). However, it may also be due to lower soil mineralisable N which has been suggested to be low in the same experimental site in previous chapters (Chapter 3, section 3.4.3). This may affect plantain N uptake and therefore availability and solubility of N in the plant. A N deficiency in plantain suggests any N taken up by the roots is utilised rather than left as luxury N that is highly soluble.

Overall, these results suggest grazing plantain has the highest potential as a forage to reduce dietary N lost to urine excretion through N solubility. This is because it contains the lowest amount of soluble N, potentially reducing the amount of ammonia converted into urea which improves N use efficiency (NUE). This result could partly explain why pastures containing plantain have been found previously to reduce urinary N concentration in cows (Box *et al.* 2016; Cheng *et al.* 2017; Totty *et al.* 2013;

Woodward *et al.* 2012). Soluble N was highest in legumes and chicory suggesting they are not a suitable feed to reduce urinary N excretion. This is because they cause excess ammonia in the rumen which, when in excess of energy to convert it to microbial protein, is easily converted into urea.

N fertiliser effect

As well as identifying the solubility of N in different forages, Goswami and Willcox (1969) showed N fertiliser rate affected N solubility in perennial ryegrass pastures therefore, could potentially influence urinary N and N losses in farm systems. Results from the current experiment confirmed this hypothesis showing NPN was greater with high N fertiliser rates than medium N fertiliser rates by up to 87 % in grasses and 159 % in herbs. This was likely due to the oversupply of soil N taken up by the plant which caused the N pool in the plant to become saturated. Consequently, excess N as nitrate and ammonia was not converted into storage proteins and instead accumulated in the plant causing a high percentage of plant NPN (Bittman and Kowalenko 2000; Hoekstra *et al.* 2008; Nowakowski and Byers 1972). Conversely, NPN in legumes was not affected by N fertiliser rate due to their ability to fix N in the soil, making N readily available for the plant to take up regardless of the soil N concentration.

NDIN was reduced at the high N fertiliser rates in grasses (420 to 340 mg/g N) and plantain (150 to 110 mg/g N) compared to the low N fertiliser rates. This suggests applying higher rates of N to the soil may increase the risk of N loss through lower insoluble N in the plant. This result was not consistent with other studies such as Coblentz *et al.* (2017) and Ledgard *et al.* (1990) who did not show differences in NDIN concentrations between low and high N fertiliser rates. However, Johnson *et al.* (2001) showed a similar decrease to the current experiment in Bermuda grass of 24 % (470 - 359 mg/g N).

Overall, a lower N fertiliser rate applied to grasses and herbs grazed by animals has the potential to decrease the proportion of highly soluble N in the diet which, in turn, could lower the risk of urinary N and nitrate leaching from a urine patch. In addition, applying lower rates of N fertiliser to forages, also decreases the risk of greater N losses through higher NDIN which is excreted in the faeces as a form of N less likely to cause N leaching.

Seasonal effect

Variation of total N between the two seasons in the current experiment agreed with previous results from other papers (Box *et al.* 2017; Cosgrove *et al.* 2007; Wilkins *et al.* 2000). Herbage N concentration and subsequent N fractions were higher in the autumn compared to summer. This was probably due to cooler autumn conditions (3 ^{o}C lower than summer) which resulted in lower herbage DM yields, lower utilisation of N in the plant and lower N dilution rates (Hoekstra *et al.* 2007; Peyraud and Astigarraga 1998). As a result, potential herbage N intake of animals would be greater in autumn which,

coupled with higher rainfall and lower plant N uptake from the soil, increases the risk of urinary N losses compared to summer.

6.4.2 WSC:CP ratio

Forage effect

It is proposed that a higher WSC:CP ratio may improve the balance of plant N and energy supply in the rumen. In turn, this may improve the utilisation of N in the animal by increasing the supply of microbial protein which is then absorbed for growth and production (Miller *et al.* 2001). This also potentially reduces the proportion of N in the diet that is lost in the urine (Edwards *et al.* 2007). To sufficiently provide microbes with enough energy to convert N into microbial protein, a threshold of 0.7 was established by Edwards *et al.* (2007). Forages with WSC:CP ratios above this threshold were deemed to have a potential to reduce N loss. The WSC:CP ratios in the current experiment was highest in diploid perennial ryegrass and plantain with values of 1.2 in the summer and 0.7 in the autumn, averaged over the N fertiliser rates. Results for perennial ryegrass in summer were consistent with other publications such as Bryant *et al* (2012) where the WSC:CP ratio was 1.2 in perennial ryegrass in the spring. However, Box *et al.* (2016) showed the WSC:CP ratio was lower in the autumn compared to the current experiment with ratios of 0.3 in perennial ryegrass and 0.5 in plantain. The reasons for the lower ratios in the study by Box *et al* (2016) is most likely due to a higher herbage CP concentration (3.6 % N, averaged) which offsets the balance of the WSC:CP ratio. In the ryegrass-white clover treatments this was due to a 25 % clover content in the treatment. However, it is unclear why higher N concentrations were also found in the 100 % plantain treatment. It is possible different harvesting methods may be the cause. In the current experiment, a cut and carry method with no animals was used. However, Box *et al.* (2016) grazed their plots with dairy cows and therefore higher N in the form of urine and faeces was applied to the soil. Overall, as plantain, chicory and diploid perennial ryegrass have WSC:CP ratios that are above the 0.7 threshold and therefore they have the potential to reduce the most N urine excretion through better balance of plant N and energy supply in the rumen.

N fertiliser effect

The WSC:CP ratio was lower at the high N fertiliser rates in diploid perennial ryegrass (0.8 at high N fertiliser rate vs 1.1 at medium N fertiliser rate) and plantain (0.6 at high N fertiliser rate vs 1.0 at medium N fertiliser rate), averaged over the two seasons. This outcome is not surprising and consistent with previous chapters (Chapter 4, section 4.3.4) and other experiments (Bryant *et al.* 2012; Coblentz *et al.* 2017; Loaiza *et al.* 2016). A declining concentration of WSC caused by a dilution effect when herbage DM yields are higher, is a common response to N fertilisation (Chapter 4, section 4.3.4), (Coblentz *et al.* 2017; Van Soest 1994) and is likely the reason for a lower WSC:CP ratio. In addition, an increase in herbage N uptake from higher N fertiliser in the soil, likely resulted in higher herbage N

concentration which offset the balance between WSC and CP in the forages. This was shown in Loaiza *et al.* (2016) where in autumn, CP concentrations increased from 17.8 % of DM at 0 kg N/ha N fertiliser to 25.0 % of DM at 450 kg N/ha which caused a decrease in WSC:CP ratios at the higher N fertiliser rate (0.94 to 0.47). Likewise, in the current study CP concentrations increased from 19.7% of DM at 180 kg N/ha N fertiliser to 23.8 % of DM at 450 kg N/ha in perennial ryegrass which caused a decrease in WSC:CP ratios at the higher N fertiliser rate (0.80 to 0.57). Overall, applying higher rates of N fertiliser lowers the herbage WSC:CP ratio which, from a N leaching perspective, is not favoured because of an inadequate supply of energy for microbes in the rumen to offset the herbage N concentration of the feed.

Seasonal effect

The WSC:CP ratio was lower in autumn with values below the 0.7 threshold (Edwards *et al.* 2007). This result probably reflects the lower herbage DM yields in cooler conditions in autumn which causes lower plant N utilisation and greater herbage N. Box *et al.* (2017) showed comparable results, with higher WSC:CP ratios in plantain and perennial ryegrass - white clover pastures in the spring, compared to autumn. Consequently, the risk of plant N lost in the rumen as urea is higher in the autumn due to lower amounts of WSC and higher amounts of herbage N which offsets the balance of the WSC:CP ratio.

6.5 Conclusions and implications

The main implications drawn from this experiment are:

- Based on soluble fractions (NPN and B1) along with the WSC:CP ratio, forages with the greatest risk of N loss are legumes, followed by chicory, grasses and lowest risk from plantain. Thus, sowing plantain as a monoculture or within a pasture mix may decrease soluble N concentration in animal diets and improve dietary N utilisation in farm systems.

- It was expected fertiliser increased the risk of poor N utilisation by increasing the solubility of N. Consequently, applying large amounts to farm systems may cause higher N losses.

- Because of greater total N concentration and a lower WSC:CP ratio of the forages in autumn, reducing fertiliser rate, grazing forages such as plantain and avoiding legumes and chicory, will reduce urinary N excretion.

Chapter 7

General discussion and conclusions

7.1 General discussion

Growing awareness of the need to decrease the environmental impacts of dairy farming (Carey *et al.* 2016; Moir *et al.* 2013; Woodward *et al.* 2012) has resulted in increasing interest in how these alternative pasture forages to perennial ryegrass and white clover could play a future in farm systems in New Zealand. In this thesis, a suite of alternative pastures to perennial ryegrass and white clover including seven grasses (diploid perennial ryegrass, tetraploid perennial ryegrass, Italian ryegrass, high sugar perennial ryegrass, tall fescue, prairie grass and cocksfoot), two herbs (chicory and plantain) and three legumes (white clover, red clover and lucerne) were analysed in a series of experiments to understand more about their role in the nitrogen (N) cycle and farm systems. This chapter will discuss the main findings of the study in relation to the hypothesis, which can be linked back to the objectives of this study (Table 1.1), with an emphasis on autumn and winter/early spring results. This is because of higher risks of nitrate leaching from lower temperatures and higher rainfall at these periods of the year (Di and Cameron 2002c).

Hypothesis #1: *In comparison to perennial ryegrass and white clover, alternate forages have greater DM yield and DM response to N.*

With concerns around poor persistence (Parsons *et al.* 2011) and lower herbage DM yields (Clark *et al.* 1996) of perennial ryegrass, there has been an interest in alternative pasture forages. The current study showed that alternative pasture forages produce higher annual herbage DM yields than traditional ryegrass and white clover under irrigation in Canterbury. This was shown in Chapter 3, Chapter 4 and Chapter 5 where forages such as plantain, prairie grass and Italian ryegrass produce up to 2244 kg DM more annual herbage DM than perennial ryegrass under the same N fertiliser regime.

Herbage DM yields of alternative pasture forages to perennial ryegrass were especially high in autumn, with up to 418 kg DM/ha, 334 kg DM/ha and 307 kg DM/ha more DM production per season in prairie grass, plantain and Italian ryegrass than perennial ryegrass in Chapter 3, averaged over the two autumn seasons and six N fertiliser rates. When multiplied by the N concentration of the forage at harvest, this equated to higher plant uptake of soil N compared to perennial ryegrass (52 kg N/ha, 51 kg N/ha and 47 kg N/ha vs 39 kg N/ha total N uptake in autumn). As a result, the potential risk of N losses may be reduced at a time of year when nitrate leaching is higher (Di and Cameron 2002c). This has been shown previously with Italian ryegrass by Malcolm *et al.* (2014) and Woods *et al.* (2016). These studies measured lower nitrate leaching from Italian ryegrass pastures compared to perennial ryegrass

pasture, reflecting the cool season growth of the Italian ryegrass. In addition, Dietz *et al.* (2013) showed plantain contained the chemical aucubin which has N inhibitory effects on soil N mineralisation thus, this could explain the increasing plant N uptake and herbage DM yield. This result suggests Italian ryegrass and prairie grass could be used as a catch crop to capture N in the soil in the winter, when nitrate leaching is likely to occur. Although there is already proof of concept for Italian ryegrass to be used as a catch crop to capture N in the soil to mitigate N losses (Carey et al. 2017) as well as other winter active forages such as oats (Carey *et al.* 2016), further field testing using lysimeters to measure nitrate leaching losses of plantain, Italian ryegrass and prairie grass over a wider range of soil and climatic conditions is warranted.

Previous experiments (Ball and Field 1982; Cameron *et al.* June 2005; O'Connor 1982) have shown DM responses to N fertiliser in perennial ryegrass often slow down at high N fertiliser rates due to saturation of plant internal N pool and the uptake of 'luxury' amounts of N from the soil. This results in inefficiency of N fertiliser use in plants and excess of N applied to the farm system that is not needed. Chapter 3 showed annual DM response rates to N fertiliser in grasses and plantain decreased after 180 kg/ha/year of N fertiliser was applied from 21.9 kg DM/kg N (180 kg N/ha/year) to 19.1 kg DM/kg N (450 kg N/ha/year). Likewise, in Chapter 4 and Chapter 5 results showed autumn DM responses to N in grasses and herbs, were higher at the medium N fertiliser (20.1 kg DM/kg N (Chapter 4) and 27.7 g DM/g N (Chapter 5)) compared to the high N fertiliser (12.5 kg DM/kg N (Chapter 4) and 20.9 g DM/g N (Chapter 5)). As a result, applying high applications of N fertiliser (above 50 kg N/ha per application or 450 kg N/ha/year) affects the ability of forages to utilise N effectively therefore increasing the risk of potential N losses in the farm system. However, an unsurprising a lack of response was shown in legumes which did not change in annual herbage DM yields with increasing rates of N fertiliser in Chapter 3 or 4 due to their ability to fix N regardless of N supply in the soil.

Low N fertiliser rates (35 kg N/ha per application or 315 N/ha/year or below) resulted in N deficiencies of the forages defined as either a decreasing quadratic DM response to N or extremely high DM responses to N. This means any stored N is depleted and no more N can be mobilised for transport around the plant for higher herbage DM yields (Williams 1955). In Chapter 3, the N response rates at low N fertiliser rate (45 kg N/ha/yr) in the tetraploid perennial ryegrass (4.9 kg DM/kg N, averaged), high sugar perennial ryegrass (16.7 kg DM/kg N, averaged) and plantain (8.3 kg DM/kg N, averaged) were lower, compared to the medium N rates (21.4 kg DM/kg N in tetraploid perennial ryegrass, 24.2 kg DM/kg N in high sugar perennial ryegrass and 21.4 kg DM/kg N in plantain at 180 kg N/ha/yr), averaged over the two years. This suggests they could not produce high DM responses until after medium N fertiliser rates of 20 kg N /ha (180 kg N/ha/year) were applied. Likewise, in Chapter 5, lower N response rates (19.7 g DM/g N) occurred at the low N treatments (2.5 g N/m^2), compared to medium N treatments (27.4 g DM/g N at 17.5 g N/m^2 fertiliser rate) suggesting soil N concentration was not

high enough to provide plant with enough N for adequate growth. The low DM response rates at low N fertiliser rates in these chapters may imply they were deficient in plant N at these treatments. Therefore, any available N in the soil is taken up and used instantly for higher herbage DM yields making it more efficient at utilising the N available rather than mobilising N from plant reserves which takes longer. Previous literature (Dairy NZ 2017; Kemp *et al.* 1999a; O'Connor 1982), shows forage DM responses to N fertiliser in grazed forages ranged between 5 – 15 kg DM/kg N. However, the current experiments were carried out using cut and carry and therefore nutrients from animal excretion were not accounted for in the experiments. This demonstrates the importance of grazing animals which provides soil with higher mineralisable N from animal's N excretion therefore, reducing the risk of N deficiencies. In addition, this result also identifies the importance of legumes such as white clover in a dairy pasture to increase herbage DM yield. Legumes have the ability to provide additional N to the grass forages through biological N fixation soil (McKenzie *et al.* 1999; Mills and Moot 2010) which allows the grasses to take up more N for plant uptake and growth (Elgersma and Hassink 1997). Thus, if the forages sown in the current trial were sown as a mixture with legumes, they may produce higher herbage DM yields because of the additional N in the soil from legume biological N fixation.

Hypothesis #2: *In comparison to perennial ryegrass and white clover, at optimal time of harvest, alternative pasture forages are lower in herbage N concentration.*

A review of the literature has shown that urinary N concentration and loading from urine patches is lower when cows ingest less N in the diet (Moorby (2014)). Thus, forages that have a low herbage N concentration have the potential to lower nitrate leaching losses from cow urine patches (Li *et al.* 2012) due to lower N loading. This is especially important in autumn when the risk of leaching is greatest because of higher rainfall (which increases drainage of soil mineral N), and cooler temperatures (which reduces plant growth and N uptake) (Di and Cameron 2002b). In Chapter 3, Italian ryegrass had the lowest autumn herbage N concentration (3.0 % of DM) at optimal time of harvest, suggesting it has the potential to reduce herbage N intake per kg DM consumed, and lead to lower N leaching losses compared to the other alternative pasture forages. This result was likely due to the high herbage DM yields in Italian ryegrass, which caused larger N dilution rates in the plant cells as DM increased (Hoekstra *et al.* 2007; Peyraud and Astigarraga 1998). Of note, in Chapter 4, plantain and diploid perennial ryegrass were also low and similar in herbage N concentration in autumn (3.0 % N), at optimum time of harvest, compared to the other alternative pasture forages. This suggests substituting perennial ryegrass for plantain into a farm system does not reduce the risk of high herbage N intake per kg DM or potential N leaching losses by reducing N loading in the urine patch. This was not expected as it had been previously suggested a lower herbage N concentration in diverse pastures containing chicory and plantain was the reason for a reduction in urinary N observed in previous studies (Beukes *et al.* 2014). This result suggests characteristics other than herbage N intake was

affecting urinary N excretion in animals fed diverse pastures, particularly those containing plantain. These include secondary plant compounds such as catapol (Deaker *et al.* 1994) and acteoside (Box *et al.* 2017; Navarrete *et al.* 2016) and higher herbage water concentration (Cheng *et al.* 2017; O'connell *et al.* 2016) which causes animals to urinate more frequently. Furthermore, as white clover was significantly higher in herbage N concentration than the grasses (4.5 % of DM vs 2.8 % of DM) this suggests that in an irrigated Canterbury environment, legumes in pastures could be a contributor to high herbage N intake by animals. However, it is important to note the significance of white clover in increasing herbage DM yield through biological N fixation in the soil (McKenzie *et al.* 1999; Mills and Moot 2010) allowing more available N for plant uptake and growth. In addition, white clover increases the pasture quality which is essential for high animal production and farm profitability (Elgersma and Hassink 1997; Harris *et al.* 1997). A typical perennial ryegrass – white clover pasture in New Zealand contain less than 20 % clover (Caradus *et al.* 1996), thus it is suggested herbage N concentrations in the sward from legumes is diluted. Therefore, in a typical ryegrass-white clover pasture, the contribution of N from legumes in the diet is low.

Previous studies have suggested herbage N intake is surplus to animal requirements when the diet contains more than 2.8 % N or 17.5 % CP (AFRC 1993; Castillo *et al.* 2001; Pacheco and Waghorn 2008). Thus, forages with more than 2.8 % N concentration pose a greater risk of N loss through the urine patch. In the current experiments, at specific times in the year regardless of the forage, herbage N concentration exceeded animal N requirements. For example, in Chapter 3, autumn herbage N concentration of over 2.8 % N at the final harvest in all the forages exceeded late lactation requirements for N of dairy cows and indicates the risk of high urinary N losses. Likewise, in Chapter 4, it was found that extending the regrowth interval to 4 weeks in autumn had little impact on the herbage N concentration, which was still over 2.8 % N at the final harvest. Therefore, further management strategies such as low N supplements should be considered to further mitigate N losses through herbage N intake.

Higher herbage DM yields in forages also equates to surplus feed per ha and more available N. This is usually managed by increasing stocking rate (SR) to balance the increase in feed supply. As a result, an increase in N leaching through higher herbage N intake per ha and number of urine patches per ha is likely to occur (Moorby 2014). This was shown in Monaghan *et al.* (2005) under a Southland dairy system, where greater herbage DM yields from higher N fertiliser rates allowed for a higher SR which caused an increase in nitrate leaching. An example of this can be demonstrated using results from Chapter 3. If two groups of cows were fed either perennial ryegrass (7544 kg DM/ha/year) or Italian ryegrass (9320 kg DM/ha/year) at 180 kg N/ha/yr N fertiliser rate, with similar annual N concentrations (2.5 – 2.6 % N). The amount of available N per ha to consume would be higher in Italian ryegrass (236 kg N/ha) than perennial ryegrass (195 kg N/ha), adding to the potential N loss in the farm system.

Nitrate leaching from high herbage DM yields were also found in Shepherd and Lucci (2013) where, N fertiliser increased herbage DM yield causing extra N consumed by animals and excreted in the urine. This is discussed in more detail in hypothesis #6.

Considering that herbage DM yield changes throughout the seasons, stocking rate could be manipulated to account for the surplus N found in farm systems at certain times of year. Therefore a change in farm management may be a sensible approach to mitigating N leaching without loss in herbage DM yield. This has been shown in Roche *et al.* (2016) where a seasonal, spring-calving, pasture-based dairy production system that imported less than 5 % of feed from off-farm and had no change in N fertiliser use, tended to decline in the amount of nitrate leached per ha with increasing stocking rate. This finding was associated with a decrease in average days in milk per cow in autumn which resulted in a lower intake of crude protein (CP) during autumn, reducing the urinary excretion of N during the most sensitive period for leaching of urine N. In addition, herbage DM yield were higher in the spring meaning there was a greater soil N uptake from plants and less N left in the soil. Furthermore, in the spring, evapotranspiration exceeded precipitation (i.e., no drainage) and therefore N leaching below the root zone could have been lower. Overall by manipulating the stocking rate to adequately suit the growing season has the potential to obtain greater plant recovery of N though the spread of urinary N may lower leaching from urine patches. Although these results do not confirm the idea of lowering the overall SR reduces N leaching, they do suggest a need to consider modifying farm management strategies around SR, in combination with forage choice, to reduce N losses per ha.

Hypothesis #3: *In comparison to perennial ryegrass and white clover, alternative pasture forages have decreased N solubility and higher WSC:CP ratio.*

It has been previously been suggested that herbage WSC:CP ratios above 0.7 are beneficial to increase the utilisation of plant N by microbes in the rumen, decreasing urinary N losses (Edwards *et al.* 2007). In Chapter 4 it was found that WSC:CP ratios after 4 weeks of regrowth in autumn were significantly higher in diploid perennial ryegrass and plantain than other alternative pasture forages (0.9 vs 0.5). With a similar ratio to perennial ryegrass, these results from Chapter 4 suggest using plantain as an alternative to perennial ryegrass will not alter N use efficiency (NUE) or effect nitrate leaching. Conversely, the low WSC:CP ratio found in white clover in autumn (0.5), may contribute to lower rumen NUE. Comparable results were shown in Chapter 6 with similar WSC:CP ratios in plantain and diploid perennial ryegrass, which were higher compared to the other forages (0.7 vs 0.5). This suggests traditional perennial ryegrass -white clover pastures likely contribute to higher urine N losses in animals.

Soluble N (non-protein N (NPN) + true protein (TP)) in the forage has been shown to contribute to high rates of urinary N concentration in a urine patch. This is due to the microbes' inability to capture such

high volumes of available N in the rumen which is instead converted into urea and lost in urine (Castillo *et al.* 2001). In Chapter 6, plantain was shown to be significantly lower in soluble N compared to diploid perennial ryegrass in autumn, with 303 mg/g N as soluble N in plantain, 475 mg/g N as soluble N in chicory and 503 mg/g N as soluble N in diploid perennial ryegrass, averaged over the two seasons and N fertiliser rates. This result could partly explain why lower urinary N concentrations were shown in cows grazing diverse pastures that contained herbs (Box *et al.* 2016; Cheng *et al.* 2017; Totty *et al.* 2013; Woodward *et al.* 2012). In addition, high soluble N in legumes in autumn (466 mg/g N) could be another reason for increasing urinary N losses in traditional perennial ryegrass -white clover pastures.

Hypothesis #4: *In comparison to perennial ryegrass and white clover, the optimum time to harvest alternative pasture forages to reduce potential leaching losses and obtain high farm production is after 4 weeks of regrowth.*

Previous research has shown that in perennial ryegrass, herbage N concentration decreases over the regrowth interval which can be explained by N dilution in the plant cells as DM increases (Hoekstra *et al.* 2007; Peyraud and Astigarraga 1998). As a result, manipulating the grazing of perennial ryegrass to obtain lower herbage N concentration therefore has the potential to reduce dietary N per kg DM given to animals. This was shown in Chapter 4, where herbage N concentration was lower in the grasses and herbs after 4 weeks compared to earlier in the regrowth interval. As a result, potential dietary N intake (per kg DM) after 4 weeks of regrowth in grasses and herbs was on average 12 % lower than dietary N intake (per kg DM) after 3 weeks of regrowth. Due to the established relationship between herbage N intake and N excretion, this may reduce the risk of higher urinary N outputs (Moorby 2014). This can be demonstrated using medium N fertiliser results from Chapter 4, with an example of a late lactation dairy cow in autumn. A cow consuming 14 kg DM/day of plantain at 3 weeks regrowth (3.4 % herbage N concentration), would consume 476 g N/cow/day. However, the same cow consuming plantain at 4 weeks regrowth (2.9 % herbage N concentration), would consume 406 g N/cow/day, potentially decreasing herbage N intake by 70 g N/cow/day. Using the relationship from Moorby (2014) (urine N output = 0.786 x diet N intake (g/day)), which showed a strong relationship between herbage N intake and urine output at herbage N intakes above 397 g N/cow/day, this would lower N excretion by 55.02 g N/day and indicate the risk of N leaching is much higher when grazing plantain at 3 weeks rather than 4 weeks. In contrast, legume herbage N concentration was unresponsive to regrowth in the autumn remaining higher compared to the other forages at 4.8 % N. This suggests legumes may contribute to higher herbage N intakes in a traditional perennial ryegrass - white clover pasture.

The ratio of WSC:CP in grasses and herbs were also highest at 4 weeks with values of up to 1.5 in spring and 0.7 in autumn, averaged over the grasses and herbs. The reason for greater WSC:CP ratio in spring may be partly due to a greater proportion of stem making up the herbage at 4 weeks, which is lower

in N % and therefore offsets the WSC:CP ratio (Hoekstra *et al.* 2007). In addition, higher herbage DM yields after 4 weeks likely caused greater N dilution in the herbage (Fulkerson and Slack 1994; Peyraud and Astigarraga 1998; Rawnsley *et al.* 2002). Herbage DM yields were substantially higher later in the regrowth with 706 kg DM/ha more herbage in week 4 than week 3 in the spring, and 322 kg DM/ha more herbage in week 4 than week 3 in the autumn, averaged over the herbs and grasses, and N fertiliser rates. In addition, WSC:CP ratios are 0.6 higher in DM in week 4 than week 3 in the spring, and 0.2 higher in DM in week 4 than week 3 in autumn, averaged over the herbs and grasses, and N fertiliser rates. As a result, to reduce the risk of N lost in the urine, the optimum time for grazing these forages is 4 weeks. However, changes in WSC:CP between regrowth intervals is not greater than selecting different forages. This is because there are larger differences in WSC:CP ratios between the forages (ranges between 0.51 in red clover to 1.66 in diploid perennial ryegrass at week 4, averaged over the seasons and N fertiliser rates), compared to differences in WSC:CP ratios between regrowth intervals (0.36 in week 1 to 0.93 in week 4, averaged over the forages, seasons and N fertiliser rates).

Herbage DM yield at 4 weeks of regrowth, averaged over the forages and N fertiliser rates, was 1772 kg DM/ha in spring, and 922 kg DM/ha in autumn. Of note, is that the quality as measured by digestible organic matter in the DM (DOMD) did not decrease as regrowth interval increased, remaining high in both spring (76.5 % of DM) and autumn (76.7 % of DM). As a result, farmers grazing alternative pasture forages at 4 weeks as a management strategy to reduce herbage N intakes and N excretion can do so without reducing herbage quality or farm production and profitability. The DOMD results were not expected because of previous research which showed DOMD would decline with an increase in regrowth interval. This is due to the accumulation of more stem and dead matter (Buxton 1996; Rawnsley *et al.* 2002). However, due to time of year, in spring and autumn and the high proportion of leaf in the sample, DOMD remained high in all the forages. Cooler temperatures in spring and autumn caused leaf appearance rates to be slower and therefore, at week 4, forages were at a leaf stage where leaf senescence had not occurred (Silsbury 1970). Also, pastures were harvested 4 cm above the ground to represent grazing height of animals. Therefore fibrous material and litter, which is lower in quality, was not measured.

Hypothesis #5: *In comparison to perennial ryegrass and white clover, lower N fertiliser rates decrease herbage N concentration of alternative pasture forages.*

Extensive research has shown as N fertiliser rate increases, herbage N concentration in forages increases in perennial ryegrass pastures (Wilkins *et al.* 2000; Moir *et al.* 2014). As a result, higher rates of N fertiliser increases dietary N concentration causing a higher risk of urinary N excretion and nitrate leaching losses from cow urine patches (Li *et al.* 2012). In all 4 experiments, as N fertiliser rate increased, herbage N concentration of herbs and grasses increased. However, this only occurred after

high amounts of N fertiliser were applied, demonstrating it was difficult to increase N concentration. For example, in Chapter 3, N concentration in plantain and grasses only increased significantly once medium rates of 35 kg N/ha/application (315 kg N/ha/year) of N fertiliser or more were applied. In addition, in Chapter 4, after 4 weeks of regrowth, N concentration of the grasses and herbs did not increase significantly until high N application rates of 50 kg N/ha/application (450 kg N/ha/year) were applied. In Chapter 5, herbage N concentrations were low until the highest N fertiliser rate of 80 kg N/ha/application (800 kg N/ha/year) was applied and finally, in Chapter 6, when higher N fertiliser rates of 50 kg N/ha/application (450 kg N/ha/year) were applied, herbage N concentration in the forages increased. These results show that from an environmental perspective, N fertiliser effects on herbage N concentration has little effect on the dietary N intake of animals and that a realistic effect of N fertiliser on the environment is from increased DM yields which increases stocking rate and therefore N loading from more urine patches.

The small effect of N fertiliser rate on herbage N concentration was also shown in Shepherd and Lucci (2013). In this previous study, the increase in pasture N concentration from autumn fertiliser was a lower risk to nitrate leaching. Instead the extra DM produced, as a result of higher N fertiliser rates, was more likely to result in extra N consumed by the animal and excreted in the urine. In the current study, this was also found and can be demonstrated using results from Chapter 3. For example, a late lactating dairy cow in autumn, consuming 14 kg DM/day of perennial ryegrass with an annual N fertiliser regime of 180 kg N/ha/year (3.0 % N), would consume 420 g N/cow/day. However the same cow consuming perennial ryegrass with an annual fertiliser regime of 315 kg N/ha/year (3.2 % N, averaged over the year), would consume 448 g N/cow/day in autumn. This potentially decreases herbage N intake in autumn by only 28 g N/cow/day. Based on the equation in Morby (2014) (urine N output = 0.786 x diet N intake (g N/day)), this would give a urinary N excretion of only 13.6 g N/cow/day less if the animals grazed the 180 kg N/ha/yr treatment over the 315 kg N/ha/yr treatment in autumn. However, due to higher herbage DM yields of diploid perennial ryegrass in the 315 kg N/ha/year N fertiliser regime (1,677 kg DM/ha in autumn) compared to the 180 kg N/ha/year N fertiliser regime (1,392 kg DM/ha in autumn), the amount of herbage N available per ha was much higher; 53.7 kg N/ha in the 315 kg N/ha/year N fertiliser regime compared to 41.8 kg N/ha in 180 kg N/ha/year N fertiliser regime, and this would contribute to higher more total losses. Thus, N fertiliser added to the potential N loss in the farm system through extra forage grown per ha may cause a higher total herbage N consumed and excreted by animals.

Legumes were mostly unresponsive to N fertiliser rate, with no significant differences between N fertiliser rates in annual herbage N concentrations in Chapter 3. In addition, in Chapter 4, after 4 weeks, there were no significant differences between N fertiliser rates in N concentration of legumes in spring and autumn, and in Chapter 6, no significant differences were shown between N fertiliser rates in N

concentration of legumes in summer and autumn. The reason for this was due their ability to fix N through biological N fixation (McKenzie *et al.* 1999; Mills and Moot 2010) which has been found previously at the same experimental site (Clement *et al.* 2016). As a result, legumes cannot be manipulated by N fertiliser to reduce herbage N intakes and lower urinary N excretion.

Hypothesis #6: *In comparison to perennial ryegrass and white clover, lower N fertiliser rates decrease N solubility and increase WSC:CP ratio of alternative pasture forages.*

Nitrogen solubility of forages has previously been shown to affect the efficiency of microbes in the rumen to convert plant N into microbial protein for animal growth and production (Bryant *et al.* 2014; Castillo *et al.* 2001; Hoekstra *et al.* 2008).

In Chapter 6, the addition of higher N fertiliser rates increased the highly soluble N concentration in grasses and herbs, increasing the risk of N losses through higher rates of N being bypassed into the urine. The reason for this was likely because of the oversupply of soil N from the high N fertiliser that caused the plant to take up luxury amounts of N after its N pool became saturated. As a result, the luxury N was converted into storage proteins and instead accumulated in the plant causing a high percentage of plant NPN (Bittman and Kowalenko 2000; Hoekstra *et al.* 2008; Nowakowski and Byers 1972). However, legume NPN concentration was not affected by higher N fertiliser because of its ability to fix its own N regardless of the soil N concentration.

WSC:CP ratios were also shown to decrease at high N fertiliser rates as shown in Chapters 4 and 6. This result was not surprising given that N concentration increased and WSC decreased offsetting the balance between the two. As a result, higher N fertiliser rates increased the risk of lower animal N use efficiency (NUE) of plant N in the rumen increasing the potential for N to be converted into urea and excreted as urinary N which is easily leached in a high rainfall event.

7.2 Limitations

Some of the limitations of this PhD research are:

- The experiments were measured over a period of two years or less and do not take into account long-term seasonal variation. Environmental effects such as differences in rainfall, temperature, climate, growing conditions, soil type and persistence are highly variable on an annual basis. Therefore, long term data collection is needed to fully understand the effects of N fertiliser and regrowth on herbage DM yield and chemical composition of alternative pasture forages.

- In all experiments, harvest dates of the grasses and herb forages was at the optimum time of harvest for perennial ryegrass, this was at the third leaf stage. However, this may not have been the optimum harvest point for other alternative pasture forages. For example, in grasses it is suggested cocksfoot is best harvested at the fourth leaf stage (Rawnsley *et al.* 2002; Turner *et al.* 2006). In addition, it has been suggested prairie grass needs a longer harvest date in spring than perennial ryegrass to allow for adequate seed setting in the summer and optimal plant survival (Fulkerson *et al.* 2000). In addition, herbs are not measured by leaf stage but rather by height, thus differences in results may also have occurred if herbs were harvested at optimum point (Lee *et al.* 2015; Li *et al.* 1997). Furthermore, some forages had larger herbage DM yields at times of year when other forages were slower to reach optimum time to harvest. For example, Italian ryegrass showed higher growth rates in the cooler months and therefore may have reached third leaf stage long before perennial ryegrass. As a result, in autumn and winter/early spring it may have been harvested past its optimum harvest point.

- In Chapter 5, the experiment was carried out in conditions that were not comparable with outdoor field experiments. Glasshouse air temperatures averaged 6^{o}C hotter that outside temperatures in the same year and therefore could affect the results.

- Also, in Chapter 5, samples were dried by an oven before being ground and analysed however, in the other experiment's samples were freeze dried. Previous research by (Dale *et al.* 2017) showed oven-drying will result in a loss of nutritive value compared to freeze drying, therefore differences chemical composition results between experiments may have occurred.

- Finally, it has been recognised that sample processing, for example; the speed of putting pasture into freezing temperatures to halt chemical processes at the time of cutting, is important. Therefore, as placing the samples in freezing temperatures straight away did not occur then this may have affected the chemical composition of the plants. This was concluded by Dale *et al.* 2017. However, it is important to note that consistency in post-harvest sampling

method is important and that within each experiment all the samples were treated in the same way post—harvest. Therefore, comparatively they are the same relative to harvest processing type. Also, it was made sure the time of day that the forages were harvested in each experiment were always the same because it is recognised that forage composition. For example; WSC and N% can change throughout the day (diurnal changes). This was always in the morning.

7.3 Conclusion and Implications

The use of specific alternative pasture forages to reduce N in the farm system is a viable mitigation option under Canterbury irrigated conditions due to their low herbage N concentration, high N use efficiency (NUE) and low NPN concentration. Moreover, medium N fertiliser rates of 20 – 35 kg N/ha per application are likely to reduce N circulating in the farm system even further. In addition, results have suggested alternative pasture forages are high in herbage DM yield compared to perennial ryegrass so can be adopted to farm systems without hindering production or profitability. The main conclusions drawn from this PhD research project are:

- The preferred forages to reduce potential N circulating from the farm system and maintain farm production are prairie grass, Italian ryegrass and plantain. This is because of their lower N concentration which reduces herbage N intake per kg DM, their higher DM response rates which reduces N fertiliser circulating the farm system, and their higher herbage DM yields in autumn and winter meaning they are able to take up more N from the soil when nitrate leaching is most likely to occur.

- Delaying grazing of grasses and herbs to 4 weeks increases herbage DM yield and WSC:CP ratios whilst reducing herbage N concentration with no compromise in quality. This was due to N dilution rates in the plant cells as DM increases, a longer period to replenish WSC in the leaf and slower leaf appearance rates.

- Grasses and herbs contained larger amounts of slower degrading protein than legumes which may be more efficiently used in the animal for growth and development rather than being lost as urea in the urine. They are therefore more suitable to reduce urinary N excretion and lower N circulating in the farm system.

- High amounts of N fertiliser applied to forages (450 kg N/ha/year or above), increased herbage N and NPN concentration, and decreased the WSC:CP ratio and N use efficiency (NUE) of forages. However, low N fertiliser rates of below 180 kg N/ha/year can significantly hinder herbage DM yield, response rates and herbage N concentration due to a nutrient deficiency. Therefore, it is suggested a medium rate of N fertiliser (180 kg N/ha/year) is the best application rate to ensure sustainable herbage DM yields without inefficient N circulating in the farm system.

7.4 Suggestions for future work

In order to have a better understanding of the nature and mechanisms of N in the farm system and to allow the transfer of this knowledge to the farming situation, further research needs to be carried out in the following areas:

- The inclusion of animals when measuring alternative pastures. Cut and carry method does not measure N from animal excretion which can contribute to up 75 % of N available for plant uptake (Nevens and Rehuel 2003; Pincay-Figueroa *et al.* 2016; Wachendorf *et al.* 2004). With this in mind, there is a greater potential for higher herbage DM yields on grazed pasture compared to cut and carry. However, there is also a larger risk of higher plant N uptake from the soil leading to greater herbage N intakes from animals which increases potential urinary N.

- The inclusion of measuring milk production in dairy cows and liveweight gain (LWG) in sheep and beef animals grazing alternative pasture forages such as plantain, prairie grass and Italian ryegrass will gain more meaningful results for farmers. Results will be much more relevant and easier adopted to farm systems, if it is proven potential profitability through increased milk solids (dairy farm) or higher carcass weights (sheep and beef farm) is higher in alternative pasture forages, compared to traditional perennial ryegrass-white clover pastures.

- Understanding the differences in managing these alternative pasture forages as diverse and monoculture pastures in a farm system may also be more meaningful to farmers. If alternative pasture forages are easily managed in a whole farm system, they are much more likely to be adopted as farmers don't need to change their system as much. Future work should include details of grazing management of individual species and mixtures.

- Measurement of herbage DM yield of alternative pasture forages such as plantain, prairie grass and Italian ryegrass for a longer period in Canterbury would clarify higher herbage DM production and persistence of alternative pasture forages to warrant farmers sowing these forages into their farm systems. In addition, animals through selective grazing may also affect persistence of forages and therefore could be investigated in the future.

- Alternative environments to irrigated Canterbury may give an insight into whether the results are true in other environments across New Zealand. This is needed to understand whether the suggested alternative pasture forages and management strategies can be used elsewhere. These conditions could include dryland vs irrigation, warmer climates for example, Waikato, different soil types for example pumice (well drained) vs gley soils (poorly drained).

- This study was inducted using monocultures not mixtures traditionally used on farms. Therefore, future research needs to be carried out to test whether DM responses to N and N concentrations are the same in mixtures.

- Finally, running a large scale farmlet experiment with subsequent treatments as alternative pasture forages, animals to graze the treatments and nitrate leaching measurements to get a more accurate understanding whether alternative pasture forages do actually reduce nitrate leaching losses.

Appendix A

Chapter 3 supplementary statistical data

Table A. 1 Linear regression analysis of dry matter (DM) yield against increasing rates of nitrogen (N) fertiliser (x) with R^2 and significance (t pr.) values.

		Year 1			Year 2		
Season	**Forage**	**Regression**	**R^2 value**	**t pr.**	**Regression**	**R^2 value**	**t pr.**
Annual	**Chicory**	9.14x + 8041	0.92	<.001	1.36x + 7515	0.12	0.295
Annual	**Cocksfoot**	20.28x + 6869	0.99	<.001	13.56x + 5749	0.99	<.001
Annual	**HSG**	18.92x + 6839	0.96	<.001	15.41x + 5714	0.99	<.001
Annual	**Italian RG**	22.31x + 4270	0.98	<.001	19.35x + 4125	0.98	<.001
Annual	**Plantain**	21.14x + 6189	0.99	<.001	20.27x + 4997	0.89	<.001
Annual	**Prairie gr**	23.94x + 7934	0.99	<.001	17.39x + 4917	0.99	<.001
Annual	**Dip PRG**	21.34x + 4342	0.99	<.001	17.17x + 3814	0.99	<.001
Annual	**Tetra PRG**	22.74x + 4557	0.97	<.001	16.47x + 4575	0.94	<.001
Annual	**Tall fescue**	18.98x + 5714	0.99	<.001	19.26x + 4412	0.99	<.001
Annual	**Lucerne**	-0.74x + 13329	0.15	0.323	-0.34x + 9855	0.01	0.820
Annual	**Red cl.**	1.98x + 12334	0.48	0.710	-1.08x + 9182	0.21	0.469
Annual	**White cl.**	5.27x + 10981	0.88	0.009	1.49x + 7812	0.60	0.322
Summer	**Chicory**	4.30x + 3966	0.85	<.001	0.40x + 3107	0.04	0.583
Summer	**Cocksfoot**	9.13x + 2908	0.98	<.001	6.00x + 2557	0.98	<.001
Summer	**HSG**	7.48x + 3446	0.89	<.001	6.18x + 2364	0.99	<.001
Summer	**Italian RG**	7.99x + 2105	0.99	<.001	9.03x + 954	0.98	<.001
Summer	**Plantain**	6.80x + 2553	1.00	<.001	8.83x + 1610	0.92	<.001
Summer	**Prairie gr**	6.99x + 4286	0.98	<.001	7.80x + 1627	0.96	<.001
Summer	**Dip PRG**	8.09x + 1956	0.99	<.001	6.55x + 978	0.98	<.001
Summer	**Tetra PRG**	8.54x + 2088	0.97	<.001	6.44x + 1396	0.98	<.001
Summer	**Tall fescue**	6.93x + 2625	0.97	<.001	7.83x + 1444	0.99	<.001
Summer	**Lucerne**	1.040x + 5577	0.26	0.407	-0.89x + 4806	0.18	0.295
Summer	**Red cl.**	-0.56x + 6621	0.06	0.651	-0.47x + 4820	0.07	0.585
Summer	**White cl.**	3.13x + 5239	0.87	0.013	0.72x + 3917	0.44	0.398
Autumn	**Chicory**	1.44x + 1338	0.82	<.001	0.25 + 942	0.21	0.473
Autumn	**Cocksfoot**	3.72x + 1248	0.99	<.001	3.14x + 691	0.87	<.001
Autumn	**HSG**	2.67x + 1254	0.92	<.001	2.79x + 578	0.94	<.001
Autumn	**Italian RG**	3.90x + 856	0.96	<.001	3.73x + 420	0.84	<.001
Autumn	**Plantain**	3.47x + 1174	0.98	<.001	3.05x + 772	0.92	<.001
Autumn	**Prairie gr**	3.87x + 1484	0.98	<.001	3.65x + 503	0.99	<.001
Autumn	**Dip PRG**	2.99x + 868	0.96	<.001	3.68x + 439	0.98	<.001
Autumn	**Tetra PRG**	4.25x + 867	0.92	<.001	3.33x + 656	0.94	<.001
Autumn	**Tall fescue**	3.27x + 1063	0.98	<.001	3.29x + 437	0.95	<.001
Autumn	**Lucerne**	0.82x + 1825	0.76	0.078	0.07x + 1206	0.00	0.866
Autumn	**Red cl.**	0.33x + 1517	0.35	0.479	-0.27x + 825	0.54	0.498
Autumn	**White cl.**	0.97x + 1631	0.70	0.038	0.46x + 932	0.38	0.244
Winter/early spr.	**Chicory**	0.85x + 606	0.74	0.031	-0.03x + 644	0.00	0.921
Winter/early spr.	**Cocksfoot**	2.32x + 693	0.99	<.001	2.01x + 336	0.99	<.001
Winter/early spr.	**HSG**	3.68x + 304	0.99	<.001	2.64x + 147	0.98	<.001
Winter/early spr.	**Italian RG**	3.80x + 290	0.91	<.001	3.00x + 76	0.93	<.001
Winter/early spr.	**Plantain**	4.28x + 1091	0.98	<.001	2.82x + 531	0.91	<.001

Winter/early spr.	**Prairie gr**	5.47x + 703	0.99	<.001	3.31x + 301	0.98	<.001
Winter/early spr.	**Dip PRG**	3.31x + 333	0.95	<.001	3.04x + 104	0.96	<.001
Winter/early spr.	**Tetra PRG**	3.69x + 369	0.84	<.001	3.16x + 208	0.91	<.001
Winter/early spr.	**Tall fescue**	3.24x + 582	0.97	<.001	3.91x + 166	0.97	<.001
Winter/early spr.	**Lucerne**	0.42x + 1603	0.39	0.354	0.50x + 893	0.43	0.110
Winter/early spr.	**Red cl.**	-0.13x + 1857	0.01	0.780	-0.14x + 980	0.05	0.656
Winter/early spr.	**White cl.**	0.61x + 1356	0.25	0.179	0.20x + 657	0.09	0.515
Late spr.	**Chicory**	2.55x + 2131	0.93	<.001	0.73x + 2821	0.19	0.192
Late spr.	**Cocksfoot**	5.11x + 2019	0.97	<.001	2.41x + 2166	0.93	<.001
Late spr.	**HSG**	5.10x + 1834	0.99	<.001	3.79x + 2678	0.76	<.001
Late spr.	**Italian RG**	6.63x + 1019	1.00	<.001	3.59x + 2675	0.98	<.001
Late spr.	**Plantain**	6.59x + 1371	0.95	<.001	5.57x + 2084	0.70	<.001
Late spr.	**Prairie gr**	7.61x + 1461	0.95	<.001	2.62x + 2485	0.86	<.001
Late spr.	**Dip PRG**	6.96x + 1186	0.93	<.001	3.91x + 2292	0.94	<.001
Late spr.	**Tetra PRG**	6.25x + 1232	0.99	<.001	3.53x + 2314	0.86	<.001
Late spr.	**Tall fescue**	5.55x + 1444	0.98	<.001	4.24x + 2365	0.93	<.001
Late spr.	**Lucerne**	-0.29x + 3329	0.05	0.680	-0.01x + 2950	0.00	0.983
Late spr.	**Red cl.**	-0.38x + 3335	0.14	0.592	-0.21x + 2557	0.08	0.744
Late spr.	**White cl.**	0.56x + 2756	0.72	0.431	0.10x + 2306	0.01	0.876

HSG, high sugar perennial ryegrass; Italian RG, Italian ryegrass; Dip PRG, diploid perennial ryegrass; Tetra PRG, tetraploid perennial ryegrass; Red cl., red clover; White cl., white clover.

Table A. 2 Quadratic regression analysis of herbage N concentration against increasing rates of N fertiliser (x) with R^2 and significance (t pr.) values.

		Year 1			Year 2		
Season	**Forage**	**Regression**	**R^2 value**	**t pr.**	**Regression**	**R^2 value**	**t pr.**
Annual	**Chicory**	$3E\text{-}06x^2 - 3E\text{-}04x + 3.20$	0.83	0.018	$2E\text{-}06x^2 + 7E\text{-}04x + 3.39$	0.87	0.213
Annual	**Cocksfoot**	$3E\text{-}06x^2 - 4E\text{-}04x + 2.93$	0.99	0.002	$4E\text{-}06x^2 - 6E\text{-}06x + 2.87$	0.99	0.007
Annual	**HSG**	$2E\text{-}06x^2 + 5E\text{-}05x + 2.40$	0.97	0.023	$2E\text{-}06x^2 + 4\ E\text{-}04x + 2.30$	0.99	0.063
Annual	**Italian RG**	$4E\text{-}06x^2 - 4E\text{-}04x + 2.33$	0.96	0.001	$4E\text{-}06x^2 + 3E\text{-}05x + 2.38$	0.97	0.003
Annual	**Plantain**	$4E\text{-}06x^2 - 1.1E\text{-}04x + 2.80$	0.98	0.001	$3E\text{-}06x^2 - 4\ E\text{-}04x + 2.66$	0.98	0.01
Annual	**Prairie gr**	$4E\text{-}06x^2 - 3E\text{-}04x + 2.26$	0.98	<.001	$3E\text{-}06x^2 + 3\ E\text{-}04x + 2.40$	1.00	0.038
Annual	**Dip PRG**	$4E\text{-}06x^2 - 6E\text{-}04x + 2.43$	0.99	<.001	$2E\text{-}06x^2 + 8\ E\text{-}04x + 2.40$	0.98	0.226
Annual	**Tetra PRG**	$4E\text{-}06x^2 - 5E\text{-}04x + 2.39$	0.97	0.001	$4E\text{-}06x^2 - 4\ E\text{-}04x + 2.46$	0.96	0.002
Annual	**Tall fescue**	$2E\text{-}06x^2 + 6E\text{-}04x + 2.54$	0.99	0.082	$3E\text{-}06x^2 + 5\ E\text{-}04x + 2.49$	1.00	0.043
Annual	**Lucerne**	$1E\text{-}06x^2 - 3E\text{-}04x + 4.31$	0.49	0.488	$3E\text{-}06x^2 - 7\ E\text{-}04x + 4.56$	0.87	0.113
Annual	**Red cl.**	$-8E\text{-}07x^2 + 4E\text{-}04x + 4.12$	0.24	0.588	$-1E\text{-}06x^2 + 4E\text{-}04x + 4.43$	0.06	0.534
Annual	**White cl.**	$4E\text{-}07x^2 + 4E\text{-}06x + 4.34$	0.25	0.765	$4E\text{-}07x^2 + 1\ E\text{-}04x + 4.54$	0.25	0.799
Summer	**Chicory**	$3E\text{-}06x^2 - 4E\text{-}04x + 2.70$	0.87	0.092	$3E\text{-}06x^2 - 4\ E\text{-}04x + 2.94$	0.72	0.149
Summer	**Cocksfoot**	$6E\text{-}06x^2 - 1.6E\text{-}04x + 2.55$	1.00	<.001	$3E\text{-}06x^2 - 4E\text{-}05x + 2.33$	0.98	0.158
Summer	**HSG**	$4E\text{-}06x^2 - 6E\text{-}04x + 2.29$	0.99	0.007	$2E\text{-}06x^2 - 1E\text{-}04x + 2.17$	0.89	0.433
Summer	**Italian RG**	$3E\text{-}06x^2 + 1E\text{-}04x + 2.14$	0.97	0.067	$2E\text{-}07x^2 - 7\ E\text{-}04x + 2.20$	0.94	0.898
Summer	**Plantain**	$5E\text{-}06x^2 - 2E\text{-}04x + 2.55$	0.96	0.003	$4E\text{-}06x^2 - 0.001x + 2.24$	0.90	0.04
Summer	**Prairie gr**	$4E\text{-}06x^2 - 4E\text{-}04x + 2.12$	0.97	0.019	$5E\text{-}07x^2 + 8\ E\text{-}04x + 2.12$	0.99	0.796
Summer	**Dip PRG**	$3E\text{-}06x^2 - 4E\text{-}04x + 2.14$	0.93	0.074	$-2E\text{-}07x^2 + 9E\text{-}04x + 2.20$	0.82	0.937
Summer	**Tetra PRG**	$5E\text{-}06x^2 - 1.5E\text{-}04x + 2.23$	0.91	<.001	$2E\text{-}06x^2 - 3\ E\text{-}04x + 2.20$	0.68	0.214
Summer	**Tall fescue**	$2E\text{-}06x^2 + 3E\text{-}04x + 2.32$	0.96	0.135	$4E\text{-}06x^2 - 8\ E\text{-}04x + 2.26$	0.98	0.05
Summer	**Lucerne**	$2E\text{-}07x^2 - 8E\text{-}05x + 3.77$	0.01	0.925	$4E\text{-}06x^2 - 1.5\ E\text{-}04x + 4.13$	0.90	0.097
Summer	**Red cl.**	$-1E\text{-}06x^2 + 4E\text{-}04x + 3.65$	0.15	0.54	$-7E\text{-}07x^2 + 3E\text{-}04x + 4.09$	0.15	0.792
Summer	**White cl.**	$-5E\text{-}07x^2 + 3E\text{-}04x + 4.07$	0.09	0.808	$2E\text{-}06x^2 - 8\ E\text{-}04x + 4.29$	0.48	0.362
Autumn	**Chicory**	$1E\text{-}06x^2 + 8E\text{-}05x + 3.60$	0.76	0.461	$3E\text{-}06x^2 - 1\ E\text{-}04x + 3.88$	0.83	0.033
Autumn	**Cocksfoot**	$2E\text{-}06x^2 - 7E\text{-}05x + 3.72$	0.97	0.15	$3E\text{-}06x^2 + 2\ E\text{-}04x + 3.42$	0.98	0.012
Autumn	**HSG**	$1E\text{-}06x^2 + 4E\text{-}04x + 3.05$	0.82	0.426	$3E\text{-}06x^2 + 2\ E\text{-}04x + 2.77$	0.99	0.028
Autumn	**Italian RG**	$4E\text{-}06x^2 - 4E\text{-}04x + 2.97$	0.99	0.011	$5E\text{-}06x^2 - 2\ E\text{-}04x + 2.68$	0.95	<.001
Autumn	**Plantain**	$2E\text{-}06x^2 - 3E\text{-}04x + 3.18$	1.00	0.15	$3E\text{-}06x^2 - 4\ E\text{-}04x + 2.95$	0.99	0.019
Autumn	**Prairie gr**	$4E\text{-}06x^2 - 2E\text{-}04x + 2.91$	0.96	0.008	$3E\text{-}06x^2 + 5\ E\text{-}04x + 2.80$	0.99	0.044
Autumn	**Dip PRG**	$5E\text{-}06x^2 - 1.2E\text{-}04x + 3.10$	0.96	0.004	$3E\text{-}06x^2 + 2\ E\text{-}04x + 2.84$	0.93	0.056
Autumn	**Tetra PRG**	$1E\text{-}06x^2 + 4E\text{-}04x + 3.03$	0.86	0.546	$3E\text{-}06x^2 + 6E\text{-}05x + 2.82$	0.93	0.037
Autumn	**Tall fescue**	$1E\text{-}08x^2 + 1.3E\text{-}04x + 3.21$	0.96	0.994	$2E\text{-}06x^2 + 1.2\ E\text{-}04x + 2.89$	1.00	0.133
Autumn	**Lucerne**	$-2E\text{-}06x^2 + 7E\text{-}04x + 4.85$	0.62	0.405	$2E\text{-}06x^2 + 1.2\ E\text{-}04x + 2.89$	0.85	0.529
Autumn	**Red cl.**	$2E\text{-}06x^2 - 7E\text{-}04x + 4.62$	0.75	0.289	$-2E\text{-}06x^2 + 7E\text{-}04x + 4.92$	0.38	0.347
Autumn	**White cl.**	$8E\text{-}07x^2 - 6E\text{-}06x + 4.71$	0.60	0.72	$-4E\text{-}07x^2 + 4E\text{-}04x + 4.94$	0.15	0.826
Wint/early sp.	**Chicory**	$2E\text{-}06x^2 - 0.0003x + 3.79$	0.54	0.201	$-2E\text{-}06x^2 + 1.9E\text{-}04x + 3.81$	0.8	0.453

Wint/early sp.	**Cocksfoot**	$9E\text{-}07x^2 + 0.0008x + 3.22$	0.98	0.63	$2E\text{-}06x^2 + 0.0003x + 3.05$	0.99	0.229
Wint/early sp.	**HSG**	$3E\text{-}06x^2 + 8E\text{-}05x + 2.49$	0.9	0.18	$2E\text{-}06x^2 + 0.0007x + 2.30$	0.97	0.243
Wint/early sp.	**Italian RG**	$3E\text{-}06x^2 - 0.0002x + 2.29$	0.68	0.118	$7E\text{-}06x^2 - 0.0011x + 2.29$	0.99	<.001
Wint/early sp.	**Plantain**	$8E\text{-}07x^2 + 0.0001x + 3.09$	0.56	0.678	$4E\text{-}06x^2 - 0.0014x + 2.88$	0.95	0.031
Wint/early sp.	**Prairie gr**	$3E\text{-}06x^2 + 0.0002x + 2.20$	0.99	0.115	$4E\text{-}06x^2 - 0.0006x + 2.42$	0.97	0.035
Wint/early sp.	**Dip PRG**	$5E\text{-}06x^2 - 0.0009x + 2.53$	0.97	0.007	$2E\text{-}06x^2 + 0.0006x + 2.37$	0.98	0.315
Wint/early sp.	**Tetra PRG**	$3E\text{-}06x^2 - 0.0001x + 2.46$	0.97	0.085	$4E\text{-}06x^2 - 0.0003x + 2.38$	0.95	0.051
Wint/early sp.	**Tall fescue**	$5E\text{-}08x^2 + 0.0013x + 2.61$	0.98	0.981	$3E\text{-}06x^2 + 0.0003x + 2.54$	0.99	0.167
Wint/early sp.	**Lucerne**	$2E\text{-}06x^2 - 0.0005x + 4.60$	0.49	0.459	$-8E\text{-}07x^2 + 1.1E\text{-}04x + 5.07$	0.69	0.776
Wint/early sp.	**Red cl.**	$-2E\text{-}06x^2 + 0.0008x + 4.28$	0.22	0.407	$-6E\text{-}06x^2 + 2.1E\text{-}04x + 4.63$	0.36	0.028
Wint/early sp.	**White cl.**	$1E\text{-}06x^2 - 0.0005x + 4.63$	0.17	0.561	$-4E\text{-}07x^2 + 4E\text{-}04x + 4.91$	0.05	0.885
Late sp.	**Chicory**	$4E\text{-}06x^2 - 0.0006x + 2.71$	0.78	0.008	$2E\text{-}06x^2 + 0.0016x + 2.91$	0.93	0.163
Late sp.	**Cocksfoot**	$4E\text{-}06x^2 - 0.0007x + 2.22$	0.99	0.006	$5E\text{-}06x^2 - 0.0004x + 2.67$	0.98	0.001
Late sp.	**HSG**	$2E\text{-}06x^2 + 0.0003x + 1.79$	0.96	0.215	$3E\text{-}06x^2 + 0.0008x + 2.15$	0.98	0.091
Late sp.	**Italian RG**	$4E\text{-}06x^2 - 0.001x + 1.92$	0.95	0.009	$4E\text{-}06x^2 + 0.0007x + 2.35$	0.95	0.019
Late sp.	**Plantain**	$7E\text{-}06x^2 - 0.002x + 2.39$	1.00	<.001	$2E\text{-}06x^2 + 0.001x + 2.56$	1.00	0.216
Late sp.	**Prairie gr**	$4E\text{-}06x^2 - 0.0006x + 1.80$	0.96	0.009	$3E\text{-}06x^2 + 0.0005x + 2.28$	0.99	0.048
Late sp.	**Dip PRG**	$2E\text{-}06x^2 + 7E\text{-}05x + 1.94$	0.98	0.235	$2E\text{-}06x^2 + 0.0015x + 2.20$	0.98	0.278
Late sp.	**Tetra PRG**	$5E\text{-}06x^2 - 0.0009x + 1.84$	0.99	0.002	$8E\text{-}06x^2 - 0.0012x + 2.38$	1.00	<.001
Late sp.	**Tall fescue**	$5E\text{-}06x^2 - 0.0004x + 1.99$	0.98	<.001	$2E\text{-}06x^2 + 0.0012x + 2.28$	1.00	0.235
Late sp.	**Lucerne**	$4E\text{-}06x^2 - 0.0012x + 4.02$	0.81	0.072	$6E\text{-}06x^2 - 0.002x + 4.24$	0.74	0.004
Late sp.	**Red cl.**	$-2E\text{-}06x^2 + 0.001x + 3.91$	0.47	0.32	$4E\text{-}06x^2 - 0.0014x + 4.07$	0.37	0.071
Late sp.	**White cl.**	$-2E\text{-}08x^2 + 0.0002x + 3.97$	0.17	0.992	$2E\text{-}07x^2 + 0.0006x + 4.01$	0.62	0.914

HSG, high sugar perennial ryegrass; Italian RG, Italian ryegrass; Dip PRG, diploid perennial ryegrass; Tetra PRG, tetraploid perennial ryegrass; Red cl., red clover; White cl., white clover.

Table A 3 Percentage dry matter (%) of twelve forage species over six nitrogen (N) fertiliser treatments over two years.

Forage	N rate	Average DM %
Chicory	0	14%
Chicory	45	13%
Chicory	90	13%
Chicory	180	13%
Chicory	350	12%
Chicory	450	12%
Cocksfoot	0	22%
Cocksfoot	45	22%
Cocksfoot	90	21%
Cocksfoot	180	21%
Cocksfoot	350	20%
Cocksfoot	450	19%
High sugar perennial ryegrass	0	24%
High sugar perennial ryegrass	45	26%
High sugar perennial ryegrass	90	23%
High sugar perennial ryegrass	180	22%
High sugar perennial ryegrass	350	21%
High sugar perennial ryegrass	450	20%
Italian ryegrass	0	21%
Italian ryegrass	45	20%
Italian ryegrass	90	20%
Italian ryegrass	180	20%
Italian ryegrass	350	18%
Italian ryegrass	450	18%
Plantain	0	16%
Plantain	45	16%
Plantain	90	16%
Plantain	180	15%
Plantain	350	14%
Plantain	450	14%
Prairie grass	0	22%
Prairie grass	45	22%
Prairie grass	90	23%
Prairie grass	180	22%
Prairie grass	350	20%
Prairie grass	450	20%
Diploid perennial ryegrass	0	23%
Diploid perennial ryegrass	45	23%
Diploid perennial ryegrass	90	23%
Diploid perennial ryegrass	180	21%
Diploid perennial ryegrass	350	20%
Diploid perennial ryegrass	450	19%
Tetraploid perennial ryegrass	0	21%
Tetraploid perennial ryegrass	45	21%
Tetraploid perennial ryegrass	90	20%

Tetraploid perennial ryegrass	180	19%
Tetraploid perennial ryegrass	350	18%
Tetraploid perennial ryegrass	450	17%
Tall fescue	0	24%
Tall fescue	45	24%
Tall fescue	90	23%
Tall fescue	180	23%
Tall fescue	350	22%
Tall fescue	450	21%
Lucerne	0	16%
Lucerne	39	17%
Lucerne	78	16%
Lucerne	156	16%
Lucerne	272	16%
Lucerne	389	16%
Red clover	0	14%
Red clover	39	15%
Red clover	78	14%
Red clover	156	15%
Red clover	272	15%
Red clover	389	15%
White clover	0	15%
White clover	45	15%
White clover	78	15%
White clover	156	14%
White clover	272	14%
White clover	389	17%

Appendix B

Chapter 4 supplementary statistical data

Table B. 1 Effects of nitrogen (N) fertiliser treatment, regrowth interval, forage and their interactions on herbage DM yield, N concentration, WSC:CP ratio and digestible organic matter in the DM (DOMD) in autumn.

N rate	Regrowth interval	Forage	Herbage DM yield (kg DM/ha)	N (% of DM)	WSC:CP ratio	DOMD (MJ/kg DM)
Nil	Week 1	Chicory	120	4.2	0.3	75.7
		Cocksfoot	108	3.6	0.2	67.5
		Plantain	140	3.4	0.3	63.1
		Red clover	132	4.7	0.2	69.9
		Diploid PRG	274	2.2	0.8	67.0
		White clover	141	4.7	0.3	77.8
	Week 2	Chicory	264	3.9	0.4	76.7
		Cocksfoot	241	3.7	0.3	69.7
		Plantain	324	3.6	0.3	70.2
		Red clover	199	4.9	0.2	69.8
		Diploid PRG	147	3.0	0.5	69.5
		White clover	284	5.0	0.5	80.3
	Week 3	Chicory	520	3.6	0.5	78.7
		Cocksfoot	326	3.6	0.3	70.6
		Plantain	570	3.2	0.5	71.7
		Red clover	518	4.6	0.3	68.4
		Diploid PRG	281	2.8	0.8	72.2
		White clover	576	4.9	0.4	79.6
	Week 4	Chicory	806	3.6	0.6	80.2
		Cocksfoot	575	3.4	0.4	71.0
		Plantain	765	3.1	1.0	77.1
		Red clover	850	4.8	0.3	73.0
		Diploid PRG	397	2.7	0.9	73.0
		White clover	809	4.7	0.5	81.0
Medium	Week 1	Chicory	118	4.1	0.2	72.7
		Cocksfoot	136	4.0	0.1	68.2
		Plantain	245	3.7	0.3	63.2
		Red clover	93	4.7	0.3	71.0
		Diploid PRG	275	3.4	0.4	70.0
		White clover	164	4.9	0.4	79.5
	Week 2	Chicory	413	4.2	0.3	76.6
		Cocksfoot	339	4.3	0.2	69.8
		Plantain	507	3.9	0.3	72.7
		Red clover	278	4.9	0.3	69.5
		Diploid PRG	284	3.4	0.5	71.6
		White clover	306	5.1	0.5	81.4
	Week 3	Chicory	718	3.8	0.5	78.9
		Cocksfoot	458	4.0	0.3	72.3
		Plantain	928	3.4	0.4	73.7
		Red clover	570	4.9	0.3	70.1

		Diploid PRG	624	3.0	0.8	75.0
		White clover	658	4.9	0.5	80.5
	Week 4	Chicory	1226	3.2	0.9	81.3
		Cocksfoot	751	3.3	0.3	69.8
		Plantain	1281	2.9	0.9	77.6
		Red clover	716	4.9	0.3	70.8
		Diploid PRG	893	2.7	1.2	76.8
		White clover	864	5.0	0.5	82.5
High	Week 1	Chicory	84	4.5	0.3	76.6
		Cocksfoot	151	4.1	0.2	67.8
		Plantain	420	3.6	0.2	66.7
		Red clover	118	4.7	0.3	71.4
		Diploid PRG	335	4.1	0.2	70.7
		White clover	179	4.6	0.4	76.1
	Week 2	Chicory	348	4.4	0.4	74.5
		Cocksfoot	358	4.7	0.3	71.1
		Plantain	715	4.3	0.5	69.9
		Red clover	322	5.1	0.3	73.2
		Diploid PRG	493	3.9	0.5	74.6
		White clover	353	4.8	0.5	81.7
	Week 3	Chicory	502	4.5	0.4	77.5
		Cocksfoot	703	4.6	0.3	73.3
		Plantain	1206	3.6	0.4	72.7
		Red clover	611	5.0	0.3	73.0
		Diploid PRG	902	3.9	0.6	76.1
		White clover	564	4.8	0.5	80.7
	Week 4	Chicory	992	4.0	0.6	80.9
		Cocksfoot	1108	4.1	0.3	73.8
		Plantain	1532	3.3	0.7	78.6
		Red clover	871	4.8	0.3	74.7
		Diploid PRG	1404	3.1	0.9	77.0
		White clover	756	4.8	0.5	81.6
		d.f	**F pr.**	**F pr.**	**F pr.**	**F pr.**
Forage (F)		5	***	***	***	***
N fertiliser rate (N)		2	***	***	NS	***
F x N		10	***	***	NS	NS
Regrowth interval (R)		3	***	***	***	***
R x F		15	**	**	***	***
R x N		6	***	NS	NS	NS
R x F x N		30	NS	NS	NS	NS

NS, not significant; Diploid PRG, diploid perennial ryegrass; d.f, degrees of freedom; F pr., significance value.

* P≤0.05
** P<0.01
***P<0.001

Table B. 2 Effects of nitrogen N fertiliser rate, regrowth interval, forage and their interactions on herbage DM yield, herbage N concentration, water soluble carbohydrate: crude protein (WSC:CP) ratio and DOMD in spring.

N rate	Regrowth interval	Forage	Herbage DM yield (kg DM/ha)	N (% of DM)	WSC:CP ratio	DOMD (% of DM)
Nil	Week 1	Chicory	124	4.0	0.4	76.1
		Cocksfoot	120	3.5	0.3	69.8
		Plantain	160	3.7	0.3	66.6
		Red clover	145	4.6	0.4	75.5
		Diploid PRG	66	2.2	1.2	70.5
		White clover	185	5.1	0.4	81.5
	Week 2	Chicory	383	3.5	0.6	75.0
		Cocksfoot	390	2.9	0.6	70.0
		Plantain	629	3.3	0.8	72.4
		Red clover	421	4.8	0.4	75.7
		Diploid PRG	148	2.5	1.4	75.4
		White clover	637	5.1	0.5	82.9
	Week 3	Chicory	769	3.1	0.6	75.7
		Cocksfoot	710	2.8	0.7	69.8
		Plantain	891	2.9	0.9	71.5
		Red clover	949	4.8	0.4	76.2
		Diploid PRG	258	2.3	1.7	75.6
		White clover	1170	4.8	0.5	82.1
	Week 4	Chicory	1455	2.7	1.2	77.8
		Cocksfoot	1098	2.3	1.2	70.1
		Plantain	1012	2.4	1.2	70.3
		Red clover	1672	4.1	0.7	76.7
		Diploid PRG	473	1.8	3.0	79.1
		White clover	1843	4.5	0.7	82.1
Medium	Week 1	Chicory	184	4.6	0.3	75.6
		Cocksfoot	172	3.6	0.3	69.4
		Plantain	146	4.1	0.3	71.2
		Red clover	179	4.8	0.4	77.3
		Diploid PRG	117	2.9	0.8	71.6
		White clover	165	5.2	0.4	82.5
	Week 2	Chicory	606	3.9	0.7	79.8
		Cocksfoot	659	3.6	0.5	73.4
		Plantain	909	3.6	0.8	74.3
		Red clover	613	4.9	0.4	75.9
		Diploid PRG	390	3.5	0.8	77.9
		White clover	582	5.1	0.5	83.6
	Week 3	Chicory	1152	3.3	0.7	77.9
		Cocksfoot	971	2.8	0.6	70.5
		Plantain	1102	3.1	0.8	71.5
		Red clover	1092	4.8	0.4	75.4
		Diploid PRG	722	2.5	1.6	78.1

		White clover	1143	4.9	0.6	83.1
	Week 4	Chicory	2087	2.6	1.3	77.5
		Cocksfoot	1906	2.2	1.2	68.7
		Plantain	2158	2.5	1.4	72.9
		Red clover	1997	4.0	0.7	75.3
		Diploid PRG	1329	2.1	2.5	79.5
		White clover	1895	4.6	0.7	82.7
High	Week 1	Chicory	268	4.3	0.3	72.9
		Cocksfoot	209	4.1	0.3	71.3
		Plantain	266	4.8	0.3	74.1
		Red clover	175	4.7	0.4	77.1
		Diploid PRG	218	3.5	0.5	72.4
		White clover	142	5.2	0.4	82.1
	Week 2	Chicory	876	4.3	0.5	78.7
		Cocksfoot	866	4.7	0.4	76.1
		Plantain	1061	4.3	0.7	78.4
		Red clover	569	5.0	0.3	75.9
		Diploid PRG	745	4.0	0.6	78.9
		White clover	649	5.1	0.5	83.8
	Week 3	Chicory	1500	4.0	0.6	78.2
		Cocksfoot	1186	3.6	0.5	72.8
		Plantain	1889	3.5	0.8	76.8
		Red clover	960	4.9	0.4	76.4
		Diploid PRG	1403	3.1	1.1	78.6
		White clover	1140	4.9	0.6	82.6
	Week 4	Chicory	2535	3.1	1.2	80.5
		Cocksfoot	2070	2.8	0.9	71.5
		Plantain	2516	2.6	1.3	77.1
		Red clover	1684	3.9	0.7	74.0
		Diploid PRG	2381	2.7	1.5	77.7
		White clover	1779	4.7	0.7	82.4
		d.f	**F pr.**	**F pr.**	**F pr.**	**F pr.**
Forage (F)		5	**	***	***	***
N fertiliser rate (N)		2	***	***	NS	***
F x N		10	***	***	***	**
Regrowth interval (R)		3	***	***	***	***
R x F		15	**	***	***	***
R x N		6	***	***	NS	NS
R x F x N		30	**	NS	NS	NS

NS, not significant; Diploid PRG*,* diploid perennial ryegrass*;* d.f, degrees of freedom; F pr., significance value.

* P≤0.05
** P<0.01
***P<0.001

Appendix C

Chapter 6 supplementary statistics

Table C. 1 Percentage of leaf, stem, and dead matter in six forage species over two nitrogen (N) fertiliser treatments (medium and high N) in summer and autumn.

Season	Forage	N fertiliser treatment	Leaf	Stem	Dead
Summer	**Diploid PRG**	Medium	95%	0%	5%
		High	97%	0%	3%
	Cocksfoot	Medium	96%	0%	4%
		High	98%	0%	2%
	Chicory	Medium	100%	0%	0%
		High	100%	0%	0%
	Plantain	Medium	100%	0%	0%
		High	100%	0%	0%
	Lucerne	Medium	80%	20%	0%
		High	82%	18%	0%
	White clover	Medium	100%	0%	0%
		High	100%	0%	0%
Autumn	**Diploid PRG**	Medium	92%	0%	8%
		High	95%	0%	5%
	Cocksfoot	Medium	92%	0%	8%
		High	94%	0%	6%
	Chicory	Medium	100%	0%	0%
		High	100%	0%	0%
	Plantain	Medium	100%	0%	0%
		High	100%	0%	0%
	Lucerne	Medium	70%	30%	0%
		High	73%	27%	0%
	White clover	Medium	100%	0%	0%
		High	100%	0%	0%
		d.f	**F pr.**	**F pr.**	**F pr.**
Forage (F)		5	***	***	***
N fertiliser rate (N)		1	***	***	***
F x N		5	**	***	***
Season (S)		1	***	***	***
F x S		5	NS	***	***
N x S		1	NS	NS	NS
F x N x S		5	NS	NS	NS

Diploid PRG; diploid perennial ryegrass; NS, not significant; d.f, degrees of freedom; F pr., significance value.

* P≤0.05

** P<0.01

***P<0.001

Table C. 2 Effect of forage, N fertiliser rate, season and their interactions on total herbage N concentration (% of DM) and N fractions of six forages

Season	Forage	N fertiliser treatment	Total N	A fraction	B1 fraction	B2 fraction	NDIN	WSC:CP
Summer	**Diploid PRG**	Medium	2.4	0.3	0.6	0.4	1.1	1.39
		High	3.1	0.6	0.6	0.7	1.2	1.06
	Cocksfoot	Medium	2.6	0.2	0.7	0.5	1.2	0.62
		High	3.3	0.3	0.7	1.0	1.3	0.49
	Chicory	Medium	2.9	0.1	1.2	1.4	0.1	1.02
		High	3.7	0.4	1.6	1.6	0.1	0.83
	Plantain	Medium	2.4	0.1	0.4	1.5	0.4	1.35
		High	3.1	0.3	0.7	1.7	0.4	1.06
	Lucerne	Medium	3.6	0.7	1.0	1.7	0.3	0.39
		High	3.6	0.8	0.8	1.6	0.3	0.43
	White clover	Medium	4.7	0.9	1.3	2.3	0.2	0.54
		High	4.5	0.7	1.2	2.3	0.2	0.60
Autumn	**Diploid PRG**	Medium	3.1	0.2	0.9	0.8	1.2	0.80
		High	4.0	0.7	1.1	1.2	1.0	0.57
	Cocksfoot	Medium	3.6	0.1	1.1	1.1	1.4	0.36
		High	4.5	0.5	1.3	1.3	1.4	0.35
	Chicory	Medium	3.4	0.1	1.5	1.7	0.2	0.61
		High	4.6	0.6	1.5	2.3	0.3	0.67
	Plantain	Medium	2.7	0.1	0.7	1.6	0.4	0.80
		High	3.9	0.4	1.0	2.1	0.4	0.73
	Lucerne	Medium	4.5	0.8	1.3	2.1	0.3	0.31
		High	4.3	0.9	1.3	1.9	0.2	0.41
	White clover	Medium	4.9	0.7	1.7	2.4	0.1	0.57
		High	4.9	0.8	1.5	2.5	0.2	0.54
		d.f	**F pr.**	**F pr.**	**F pr.**	**F pr.**	**F pr.**	**F pr.**
Forage (F)		5	***	***	***	***	***	***
N fertiliser rate (N)		1	***	***	***	***	NS	**
F x N		5	***	**	**	**	NS	*
Season (S)		1	***	NS	***	***	NS	***
F x S		5	NS	NS	NS	NS	NS	**
N x S		1	NS	**	NS	NS	NS	NS
F x N x S		5	NS	NS	NS	NS	NS	NS

A, non protein N; B1, soluble true protein; B2, insoluble protein, neutral detergent soluble true protein; NDIN, neutral detergent insoluble protein; Diploid PRG, diploid perennial ryegrass; NS, not significant; d.f, degrees of freedom; F pr., significance value.

* P≤0.05
** P<0.01
***P<0.001

Table C. 3 Effect of forage, N fertiliser rate, season and their interactions of N fractions when expressed as mg/g N of six forages.

Season	Forage	N fertiliser treatment	A fraction	B1 fraction	B2 fraction	NDIN
Summer	**Diploid PRG**	Medium	130	240	170	460
		High	180	200	240	390
	Cocksfoot	Medium	90	260	190	460
		High	100	200	310	400
	Chicory	Medium	50	410	500	40
		High	110	420	440	30
	Plantain	Medium	50	180	620	150
		High	110	230	550	120
	Lucerne	Medium	190	260	460	90
		High	230	230	460	80
	White clover	Medium	190	270	500	40
		High	160	270	510	50
Autumn	**Diploid PRG**	Medium	70	280	270	380
		High	180	260	300	260
	Cocksfoot	Medium	20	300	300	380
		High	120	290	280	320
	Chicory	Medium	40	430	480	40
		High	120	320	490	60
	Plantain	Medium	30	240	580	150
		High	100	270	530	100
	Lucerne	Medium	170	300	480	60
		High	200	310	440	50
	White clover	Medium	130	350	490	30
		High	170	300	500	30
		d.f	**F pr.**	**F pr.**	**F pr.**	**F pr.**
Forage (F)		5	***	***	***	***
N fertiliser rate (N)		1	***	**	NS	***
F x N		5	*	NS	NS	**
Season (S)		1	NS	***	NS	***
F x S		5	NS	NS	NS	***
N x S		1	**	NS	NS	NS
F x N x S		5	NS	NS	NS	NS

A, non- protein N; B1, soluble true protein; B2, insoluble protein, neutral detergent soluble true protein; NDIN, neutral detergent insoluble protein; Diploid PRG; diploid perennial ryegrass; NS, not significant; d.f, degrees of freedom; F pr., significance value.

* P≤0.05
** P<0.01
***P<0.001

Appendix D

Published papers

Herbage dry-matter yield and nitrogen concentration of grass, legume and herb species grown at different nitrogen-fertiliser rates under irrigation

Kirsty Martin[A,C], *Grant Edwards*[A], *Rachael Bryant*[A], *Miriam Hodge*[A], *Jim Moir*[A], *David Chapman*[B] *and Keith Cameron*[A]

[A]Faculty of Agriculture and Life Sciences, PO Box 85084, Lincoln University. Lincoln 7647, Christchurch, New Zealand.

[B]DairyNZ, Canterbury Agriculture and Science Centre, Gerald Street, PO Box 85066, Lincoln 7647, Christchurch, New Zealand.

[C]Corresponding author. Email: Kirsty.martin@lincolnuni.ac.nz

Abstract. An important goal in dairy systems is to increase production while achieving environmental targets associated with lower nitrate leaching from soils. One approach is to identify forages that grow more at a given level of nitrogen (N) input and result in a lower N intake per kilogram dry matter (DM) consumed. However, while N responses have been well described for perennial ryegrasses, less information is available for alternative grasses, legumes and herbs. In the present study, conducted on the Canterbury Plains, New Zealand, six species (perennial ryegrass, Italian ryegrass, white clover, lucerne, chicory and plantain) were grown at six N-fertiliser rates ranging from 0 to 450 kg N/ha. year and managed under irrigation and cutting management. Herbage DM yield and N concentration were measured over 12 months. As N-fertiliser rate increased from 0 to 450 kg N/ha, annual herbage yield increased linearly (from 4794 to 14 329 kg DM/ha.year in grasses and from 7146 to 13 177 kg DM/ha.year in herbs). In contrast, annual herbage yield for legumes was unaffected by N-fertiliser rate and ranged from 11 874 to 13 112 kg DM/ha. Additionally, there were contrasting responses in herbage N concentration between species. At all N-fertiliser rates, herbage N concentration was highest in legumes (43.4 g N/kg DM), then herbs (30.7 g N/kg DM), and lowest in grasses (25.8 g N/kg DM). The N concentration of legume DM was unaffected by increasing N-fertiliser rate, whereas in grasses and herbs it increased. Results suggested that there were no benefits in using herbs instead of grasses for reducing N intake in livestock in an irrigated Canterbury environment.

Additional keywords: chicory, clover, Italian ryegrass, lucerne, nitrate leaching, plantain. Received 15 July 2016, accepted 21 December 2016, published online 31 March 2017

Introduction

Regulations that place a limit on the amount of nitrate leaching from agricultural land are currently being developed by Regional Councils throughout New Zealand (Ministry for the Environment 2014). These regulations may require substantial reductions in nitrate leaching from current typical dairy-farm levels and approaches are sought to achieve these reductions, while sustaining or improving profitability (Bryant *et al*. 2007).

The urine patch is the primary source of leached nitrate in pasture-based dairy systems (Di and Cameron 2002). The main factor influencing the amount of nitrogen (N) excreted in urine is the amount of N consumed by animals relative to the demands of production, maintenance and body tissue retention (Kebreab *et al*. 2001). Therefore, a logical pathway to controlling N surplus in the animal (the amount of N ingested minus amount required) is to manipulate the N concentration in the feeds they are eating, provided intakes are the same. One approach is to identify forages that result in a lower N intake per kilogram dry matter (DM) consumed. However, while N fertiliser-rate trials have improved our knowledge of the relationships between soil N supply, DM yield and N composition of herbage in some grasses (perennial ryegrass (Hill *et al*. 2005) and cocksfoot (Mills *et al*. 2009)), less information is available for alternative herbs and legume species (e.g. plantain, chicory, lucerne and white clover). This information is necessary to identify candidate pasture species for future forage systems that lead to lower nitrate- leaching losses.

The objective of the experiment was to compare the effect of N-fertiliser application on herbage DM yield and N concentration of two grass species (perennial ryegrass, Italian ryegrass), two legume species (white clover and lucerne) and two herb species (chicory and plantain) over 1 year.

Materials and methods

Experimental site and design

The experiment was conducted from 1 December 2014 to 30 November 2015 at the Lincoln University Research Dairy Farm, Canterbury, New Zealand (43°64′S, 172°46′E). The soil type was a free-draining Templeton fine sandy loam (Immature Pallic soil; Hewitt 2010). The experiment consisted of four replicates of a split-plot factorial design, with six forage species as the main plot treatments and six N-fertiliser rates as subplot treatments. The six species and their sowing rates are shown in Table 1. The N-fertiliser rates were 0, 45, 90, 180, 315 and 450 kg N/ha.year for grasses and herbs, and 0, 39, 78, 156, 272 and 389 kg N/ha.year for legumes. The main plots were 3 m · 12.6 m, and subplots 3 m · 2.1 m. All species were sown as pure swards.

Management

The site was sprayed with glyphosate, cultivated with a Duncan Contoura with rear crumbler, power-harrowed using a LELY Power Harrow (2.5 m width) and rolled with a Cambridge Roller in March 2014. The plots were sown with a Flexiseeder 14-row plot drill (width 2.1 m) on 20 March 2014. Soil nutrient sampling was conducted before the experiment with a soil corer to a depth of 75 mm; results showed pH = 6.1 (soil : water ratio, 1 : 2), Olsen phosphorus (P) = 26 mg/L (Olsen *et al*. 1954), sulfate-sulfur (sulphate-S) = 5 mg/kg (Watkinson and Kear 1994) and potassium (K) = 0.23 milliequivalents/100 g (Rayment and Higginson 1992). On the basis of this, plots were fertilised as shown in Table 2, to ensure that these nutrients were not limiting to the pasture species under investigation. Due to the harvest method (cut and carry), higher levels and more frequent applications of potassium fertiliser was applied to the lucerne plots to keep up with greater nutrient demands (Harris *et al*. 1966; Metson 1974).

Herbicide T Max (40 mL/10 L water) was applied to grass and plantain plots on 22 January and 4 May 2015 to remove dicotyledon species, particularly white clover. Herbicide Gallant (5 mL/10 L water) with surfactant Uptake (50 ml/20 L water) was applied to legume and herb plots on 4 December 2014, 22 January, 4 May and 10 June 2015 to remove grass species from legume and herb plots.

The site was irrigated with a travelling irrigator between October and March, with ~20–30 mm water applied per week (total 550 mm). Plots were allowed to establish in the absence of defoliation until spring 2014, and they were cut and fertilised at the beginning of October and November before the trial began. The first trial harvest to be conducted was on 1 December 2014. The timing of defoliation was determined by best practices (Moot *et al*. 2003; Lee *et al*. 2011) for maximising herbage growth and persistence in the three different functional groups, namely, grasses, legumes and herbs. Grasses and herbs were defoliated at 32-, 26- and 30-day intervals in spring, summer and autumn respectively. Legumes were harvested at 41-, 35- and 41-day intervals in spring, summer and autumn respectively. Due to low soil temperatures and slow growth rates, no plots were harvested in winter. Herbage was defoliated and removed from each plot with a Walker MC GHS (20 HP, 14.9 kW) ride on rotary lawnmower; the mower height was set to 4 cm for all species. N fertiliser was applied following each defoliation as calcium ammonium nitrate (27 : 0 : 0 : 0; N : P : K : S), with the total annual N application rate split evenly throughout the year. Before the experiment began, the planned N-fertiliser treatments were 0, 50, 100, 200, 350 and 500 kg N/ha.year. It was estimated that 9 (legumes) or 10 (grasses and herbs) harvests would occur and fertiliser application rates were calculated on this basis. However, due to best practice methods, plots were harvested fewer times than expected, which resulted in nine harvests for grasses and herbs and seven harvests for legumes over the period from 1 December 2014 to 30 November 2015. This led to in lower levels of N applied than proposed and differences in rates of N fertiliser applied between legumes and grasses and herbs.

Table 1. Forage species sown and their functional group, scientific name, cultivar and sowing rate (kg/ha)

Forage	Functional group	Scientific name	Cultivar	Sowing rate (kg/ha)
Perennial ryegrass	Grass	*Lolium perenne*	One 50 AR37	20
Italian ryegrass	Grass	*Lolium multiflorum*	Tabu, diploid	25
White clover	Legume	*Trifolium repens*	Kopu II	5
Lucerne	Legume	*Medicago sativa*	Torlesse	14
Chicory	Herb	*Cichorium intybus*	Choice	8
Plantain	Herb	*Plantago lanceolata*	Tonic	10

Herbage measurements

At each harvest, one 3 m · 0.45 m strip was cut with a Briggs & Stratton 650 series 190cc rotary blade push mower to a height of 4 cm and herbage collected in the catcher. The fresh weight of herbage was recorded and two subsamples of ~50–100 g fresh weight were then taken. The first subsample was weighed fresh and oven-dried at 60^{O}C for 48 h to determine DM% (Adesogan *et al*. 2000). The DM% was multiplied by fresh weight to determine herbage yield. The second subsample was frozen and freeze-dried before being ground through a 1-mm sieve with a M200 rotor mill (Retsch Inc., Newtown, PA, USA) and scanned by near- infrared spectrophotometry (NIRS, NIRSystems 5000, Foss, MD, USA) to determine N concentration. The NIRS results were calculated using plant species from the current study. However, when samples were outside the calibration spectrum, wet chemistry was performed by combustion under oxygen (Elemental Analyser vario MAX CN, Analysensysteme GmbH, Hanau, Germany). Data for each plot were averaged across the year for statistical analysis.

Statistical analyses

Herbage DM yield and N concentration on annual basis were analysed by multiple linear regression with groups (species), using the statistical package GENSTAT, version 16 (VSN International 2013), by using the following model:

$$y = \alpha_j + \beta_j x_j + e_j,$$

where y is independent variable (DM yield or N concentration), a is the overall mean, b is the block, j is the plant species, x is N- fertiliser rate and e is the residual error. Average N concentration was calculated using the mean number of harvests and N concentration averaged across the year.

Results

Climate data

Annual air temperature was 0.5°C higher than the long-term average (Fig. 1*a*). Rainfall was generally below the long-term average in the key growing seasons of spring, summer and autumn (Fig. 1*b*) In 2014–2015, rainfall was 65% of the long- term average. This was compensated by irrigation, which was applied at a rate of 23 mm/week, supplemental to rainfall, between October and March.

Herbage DM yield

There was a significant ($P < 0.001$) interaction between N- fertiliser rate and species for annual herbage DM yield; this was particularly noted at lower N rates (Fig. 2). Annual herbage yield of the two legumes was unaffected by N-fertiliser rate. However, in the other four species, annual herbage yield increased linearly with each N-fertiliser rate (Fig. 2), with plantain and Italian ryegrass being very similar in their response to N-fertiliser rate.

Table 2. Total nutrients applied (kg/ha) to each forage species

Date	Species	Total nutrients applied (kg/ha)			
		Phosphorus	Potassium	Sulphur	Lime
Mar. 2014	All species	12.8	20	32.8	0
Oct. 2014	All species	12.8	20	32.8	2000
Dec. 2014	Lucerne	28	75	72	2000
Sep. 2015	Lucerne	45	250	55	0

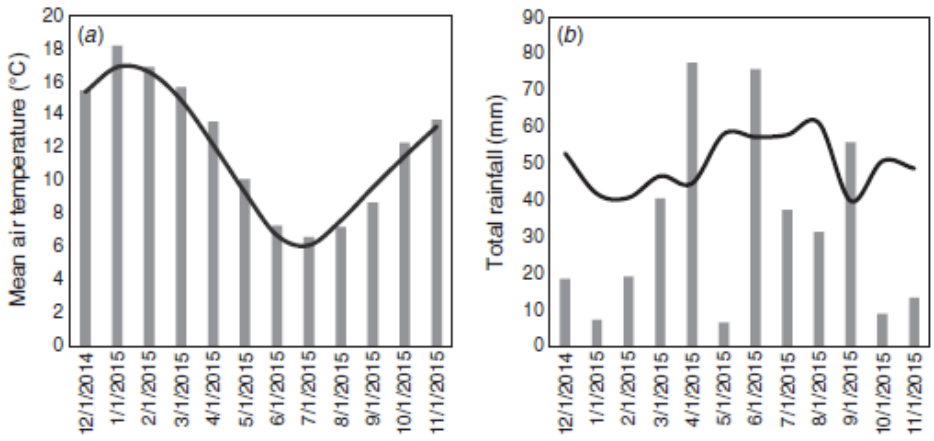

Fig. 1. (*a*) Monthly mean air temperature and (*b*) mean monthly rainfall for December 2014–November 2015 at Lincoln, Canterbury, New Zealand. Data were collected from Broadfields Meteorological Station,1 km from research site. December 2014–November 2015 (—), long-term average (1981–2010) (—).

Averaged across all N-fertiliser rates, annual herbage DM yield was greater ($P < 0.001$) in lucerne (12 548 kg DM/ha.year) and white clover (11 801 kg DM/ha.year) than in plantain (10 069 kg DM/ha.year), chicory (9628 kg DM/ha.year) and Italian ryegrass (9994 kg DM/ha.year), being the

lowest in perennial ryegrass (7702 kg DM/ha.year; Fig. 2). Perennial ryegrass yielded the least at all N rates except 450 kg N/ha.year (Fig. 2). However, when comparing maximum yield potential (450 kg N/ha.year) among all the species, plantain and Italian ryegrass were the highest-yielding pastures (14 803 and 15 232 respectively) and yielded more than did the legumes (white clover 13 391 kg DM/ha, lucerne 12 833 kg DM/ha).

N concentrations in plants

There was a significant interaction between N-fertiliser rate and species for average N concentration of the herbage (Fig. 3). The N concentration of the two legumes was unaffected by N fertiliser. However, in other species, N concentration increased with an increasing N-fertiliser rate. (Fig. 3). Averaged across all N-fertiliser rates, N concentration was greater ($P < 0.001$) in lucerne (43.1 g/kg DM) and white clover (43.7 g/kg DM) than in plantain (28.7 g/kg DM) and chicory (32.7 g/kg DM), with Italian ryegrass (25.7 g/kg DM) and perennial ryegrass (26.0 g/kg DM) having the lowest N concentration (Fig. 3).

Discussion

DM yield

Across all fertiliser rates, a higher annual DM yield occurred in legumes than in grasses, with legumes producing 4473 kg DM/ha more annual herbage than does perennial ryegrass. However, at high N rates, grasses yielded more than legumes (14 329 kg DM/ ha.year at 450 kg N/ha.year for grasses verses 13 112 kg DM/ha. year at 389 kg N/ha.year for legumes). High summer growth rates (data not shown) were the key reason behind high legume DM yields. Consistent with the results of Orr et al. (1990), herbage DM yield in legumes was greater than in grass and herb species at all N-fertiliser rates up to 315 kg N/ha. The growth potential of lucerne at low fertiliser rates over perennial ryegrass and herbs has been well documented under both irrigated and dryland conditions (Brown et al. 2005; Hayes et al. 2010; Mills and Moot 2010). However, what has often been overlooked is the high DM yield of white clover monocultures compared with ryegrass monocultures. Brock (1973) found that white clover yield was 9760–12 010 kg DM/ha.year under dryland conditions. This illustrated the ability of legumes to fix their own N as well as growing rapidly in the summer, where rainfall is adequate.

Fertiliser rates

Contrasting effects of N fertiliser were found on DM yield, with grasses and herbs showing a positive response, while legumes were unresponsive. The average N response in grasses and herbs across all N rates was 21.1, 24.4, 19.8 and 9.5 kg DM/ kg N for

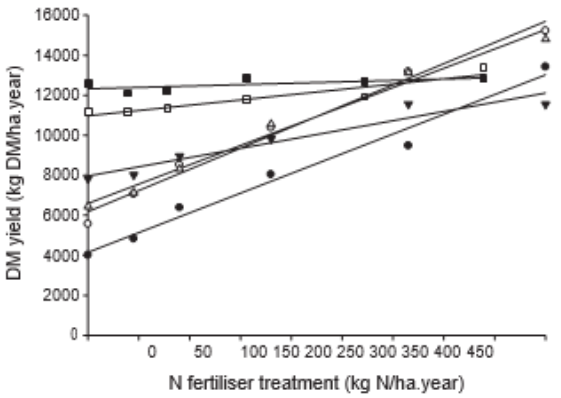

Fig. 2. Effect of nitrogen-fertiliser rate (kg N/ha) on annual herbage dry- matter (DM) accumulation (kg DM/ha.year) for the following six pasture species: perennial ryegrass (●), Italian ryegrass (○), chicory (⍰), plantain (Δ), lucerne (■) and white clover (□). Regression of each species is as follows: perennial ryegrass $y = 19.715x + 4153.4$ ($R^2 = 0.9778$), Italian ryegrass $y = 21.141x + 6188.9$ ($R^2 = 0.9843$), chicory $y = 9.2077x + 7970.8$ ($R^2 = 0.9257$), plantain $y = 19.307x + 6594.4$ ($R^2 = 0.9861$), lucerne $y = 1.3956x + 12331$ ($R^2 = 0.4615$) and white clover $y = 5.3393x + 10976$ ($R^2 = 0.8807$).

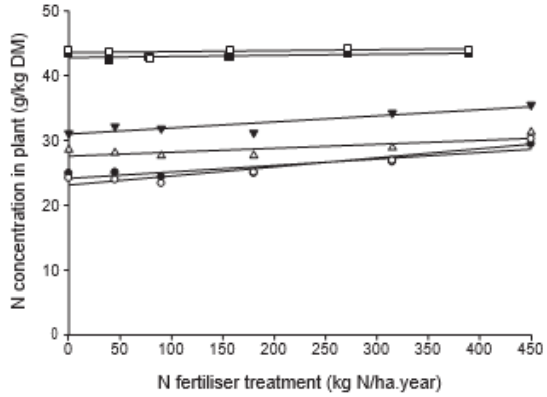

Fig. 3. Effect of nitrogen (N)-fertiliser rate (kg N/ha) on N concentration (g/kg DM) of herbage harvested averaged across the year for the following six pasture species: perennial ryegrass (●), Italian ryegrass (○), chicory (⍰), plantain (Δ), lucerne (■) and white clover (□). Regression of each species is as follows: perennial ryegrass $y = 0.001x + 2.4162$ ($R^2 = 0.8199$), Italian ryegrass $y = 0.0014x + 2.3145$ ($R^2 = 0.8908$), chicory $y = 0.0009x + 3.1011$ ($R^2 = 0.8153$), plantain $y = 0.0006x + 2.7607$ ($R^2 = 0.6116$), lucerne $y = 0.0002x + 4.2807$ ($R^2 = 0.3381$) and white clover $y = 0.0002x + 4.3549$ ($R^2 = 0.1626$).

perennial ryegrass, Italian ryegrass, plantain and chicory respectively. The average N DM response of chicory was lower than expected (Clark *et al*. 1990; Collins and McCoy 1997). The reason for this is unclear, but may reflect the higher DM yield of chicory at a low N input in the current study than what has been reported earlier. With no N fertiliser, annual yield of chicory was 7836 kg DM/ha and greater than that of perennial ryegrass (+3820 kg DM/ha), Italian ryegrass (+2268 kg DM/ha) and

plantain (+1384 Kg DM/ha). Further, studies in mixed pastures at the same site showed a lower response of diverse pastures (which contained less than 20% chicory) to N fertiliser than that of standard perennial ryegrass–white clover pasture (Van Rossum *et al*. 2013). Alternatively, the lower N response in chicory could have been due to cutting interval, which was 5–6 weeks in previous studies compared with 4 weeks in the current study. The lack of an effect of N fertiliser on legumes has been noted previously and reflects the ability of legumes to fix N for their N requirements (McKenzie *et al*. 1999). Legumes may use N from N fertiliser when available rather than from N fixation (Schwinning and Parsons 1996), which means that total N in the plant maybe unaltered. However, it is important to note that N fixation in legumes will not completely cease when N fertiliser is adequate for plant growth (Armstrong *et al*. 1999). An increase in yield from applied fertiliser N has been found in irrigated and unirrigated lucerne and white clover swards (Cowling 1961; Hoglund *et al*. 1974). Previous studies have shown that an N response was due to lack of rhizobia in the soil (Hoglund *et al*. 1974), cold temperatures which supresses N fixation in early spring (Hoglund *et al*. 1979; Williams 1932; Young 1958) or soils being low in organic matter (Hannaway and Shuler 1993).

N concentration

The N concentration of herbage was greatest in legumes, intermediate in herbs and lowest in grasses. The higher N concentration of legumes versus grasses and herbs at all N rates is consistent with the results of previous studies (Fraser and Rowarth 1996; Elgersma and Hassink 1997).While this high N concentration in legumes is important for pasture growth, it does contribute to a high N intake by cows grazing mixed pastures, with consequent high rates of N excretion in urine (Kebreab et al. 2001). Previous short-term studies by Totty et al. (2013) and Woodward et al. (2012) have shown that feeding diverse pastures containing herbs (chicory, plantain) in addition to perennial ryegrass–white clover, can result in lower urinary N excretion from dairy cows in late lactation than does feeding perennial ryegrass–white clover. This was proposed to be because herbs have a lower N concentration, thus reducing the N intake of a diverse pasture (Beukes et al. 2014). However, in the current study, conducted under irrigation, herbage N concentrations in chicory and plantain were higher than in Italian ryegrass and perennial ryegrass. In turn, this may lead to higher N intakes per unit of DM consumed in herbs than grasses. This indicates that factors other than N intake may drive lower N excretion in livestock grazing pasture containing herbs (Stewart 1996; Tamura and Nishibe 2002; Box et al. 2016).

There were contrasting effects of N fertiliser on the N concentration for the different species; legumes were unresponsive to N fertiliser, whereas the N concentration of herbs and grasses increased, although the increase was small. This may have depleted soil N, so that although herbage DM yield increased with low rates of N application, an increase of N concentration did not occur until relatively high rates of N were applied. The average N concentration was higher at all N-fertiliser rates in plantain and chicory (28.7 and 32.7 g N/kg DM respectively) than in ryegrass (26 g N/kg DM), which is consistent with the results of previous studies (Collins and McCoy 1997; Belesky et al. 2000; Sanderson et al. 2003). This may reflect an improved N uptake by these plants with deeper and more vigorous rooting systems. Alternatively, it may be a consequence of the harvesting regime, which was similar for grasses and herbs. The N concentration of herbage declines with regrowth period in perennial ryegrass (Nowakowski 1962; Chaves et al. 2006) However, it is unclear whether the decline is similar in herbs.

Implications

Legumes had a higher DM yield than did grasses and herbs at fertiliser rates up to 272 kg N/ha.year, but did not respond to N fertiliser. At all N-fertiliser rates, the N concentration was highest in legumes, intermediate in herbs and lowest in grasses. The results suggested that for the irrigated Canterbury environment, legumes in pastures could be a contributor to high N intake by animals and that there were no large benefits from using herbs instead of grasses in reducing N intake of livestock when DM intakes are the same.

Acknowledgements

This research was undertaken as part of the Forages to Reduced Nitrate Leaching program, with principal funding form the New Zealand Ministry of Business, Innovation and Employment. The program is a partnership between DairyNZ, AgResearch and Landcare Research.

References

Adesogan AT, Givensz DI, Owen E (2000) Measuring chemical composition and nutritive value in forages. In 'Field and laboratory methods for grassland and animal production research'. (Eds L t'Manneje, RM Jones) pp. 263–275. (Cambridge University Press: Cambridge, UK)

Armstrong R, McCosker K, Johnson S, Millar G, Walsh K, Kuskopf B, Probert M, Standley J (1999) Legume and opportunity cropping systems in central Queensland. 1. Legume growth, nitrogen fixation, and water use. Crop & Pasture Science **50**, 909–924. doi:10.1071/ AR98100

Belesky DP, Turner KE, Ruckle JM (2000) Influence of nitrogen on productivity and nutritive value of forage chicory. Agronomy Journal **92**, 472–478. doi:10.2134/agronj2000.923472x

Beukes PC, Gregorini P, Romera AJ, Woodward SL, Khaembah EN, Chapman DF, Nobilly F, Bryant RH, Edwards GR, Clark DA (2014) The potential of diverse pastures to reduce nitrogen leaching on New Zealand dairy farms. Animal Production Science **54**, 1971–1979.

Box L, Edwards GR, Bryant RH (2016) Milk production and urinary nitrogen excretion of dairy cows grazing perennial ryegrass–white clover and pure plantain swards. Proceedings of the New Zealand Society of Animal Production **76**, 18–21.

Brock J (1973) Growth and nitrogen fixation of pure stands of three pasture legumes with high/low phosphate. New Zealand Journal of Agricultural Research **16**, 483–491. doi:10.1080/00288233.1973.10421093

Brown HE, Moot DJ, Pollock KM (2005) Herbage production, persistence, nutritive characteristics and water use of perennial forages grown over 6 years on a Wakanui silt loam. New Zealand Journal of Agricultural Research**48**,423–439.
doi:10.1080/00288233.2005.9513677

Bryant JR, Hoogendoorn CJ, Snow VO (2007) Simulation of mitigation strategies to reduce nitrogen leaching from grazed pasture. In 'Proceedings of the 69th New Zealand Grassland Association Conference', Wairakei, New Zealand. pp. 145–151. (The New Zealand Grassland Association: Dunedin, New Zealand)

Chaves AV, Waghorn GC, Brookes IM, Woodfield DR (2006) Effect of maturation and initial harvest dates on the nutritive characteristics of ryegrass (Lolium perenne L.). Animal Feed Science and Technology **127**, 293–318. doi:10.1016/j.anifeedsci.2005.08.015

Clark DA, Anderson CB, Berquist T (1990) Growth rates of 'GrasslandsPuna' chicory (Cichorium intybus L.) at various cutting intervals and heights and rates of nitrogen. New Zealand Journal of Agricultural Research **33**, 213–217. doi:10.1080/00288233.1990.10428412

Collins M, McCoy JE (1997) Chicory productivity, forage quality, and response to nitrogen fertilization. Agronomy Journal**89**,232–238.
doi:10.2134/agronj1997.00021962008900020013x

Cowling D (1961) The effect of nitrogenous fertilizer on an established white clover sward. Grass and Forage Science **16**, 65–68. doi:10.1111/j.1365- 2494.1961.tb00213.x

Di HJ, Cameron KC (2002) Nitrate leaching in temperate agroecosystems:
sources, factors and mitigating strategies. Nutrient Cycling in Agroecosystems **64**, 237–256. doi:10.1023/A:1021471531188

Elgersma A, Hassink J (1997) Effects of white clover (Trifolium repens L.) on plant and soil nitrogen and soil organic matter in mixtures with perennial ryegrass (Lolium perenne L.). Plant and Soil **197**, 177–186. doi:10.1023/A:1004237527970

Fraser TJ, Rowarth JS (1996) Legumes, herbs or grass for lamb performance? In 'Proceedings of the 58th New Zealand Grassland Association Conference', Oamaru, New Zealand. pp. 49–52. (The New Zealand Grassland Association: Dunedin, New Zealand)

Hannaway D, Shuler P (1993) Nitrogen fertilization in alfalfa production. Journal of Production Agriculture **6**, 80–85. doi:10.2134/jpa1993.0080 Harris PB, McNaught KJ, Lynch PB (1966) The main effects and interactions of nutrients on the growth of irrigated lucerne. New Zealand Journal of Agricultural Research **9**, 653–690. doi:10.1080/
00288233.1966.10431556

Hayes RC, Dear BS, Li GD, Virgona JM, Conyers MK, Hackney BF, Tidd J (2010) Perennial pastures for recharge control in temperate drought- prone environments. Part 1: productivity, persistence and herbage quality of key species. New Zealand Journal of Agricultural Research **53**, 283–302. doi:10.1080/00288233.2010.515937

Hewitt AE (2010) 'New Zealand soil classification.' (Manaaki Whenua Press: Lincoln, New Zealand)

Hill JO, Simpson RJ, Wood JT, Moore AD, Chapman DF (2005) The phosphorus and nitrogen requirements of temperate pasture species and their influence on grassland botanical composition. Australian Journal of Agricultural Research **56**, 1027–1039. doi:10.1071/AR04279

Hoglund J, Dougherty C, Langer R (1974) Response of irrigated lucerne to defoliation and nitrogen fertiliser. New Zealand journal of Experimental
Agriculture**2**,7–11.
doi:10.1080/03015521.1974.10427661

Hoglund JH, Crush JR, Brock JL, Ball R, Carran RA (1979) XII. General discussion. New Zealand journal of ExperimentalAgriculture**7.**45–51.
doi:10.1080/03015521.1979.10426162

Kebreab E, France J, Beever DE, Castillo AR (2001) Nitrogen pollution by dairy cows and its mitigation by dietary manipulation. Nutrient Cycling in Agroecosystems **60**, 275–285. doi:10.1023/A:1012668109662

Lee J, Hedley P, Roche J (2011) Grazing management: guidelines for optimal pasture growth and quality. Dairy NZ Technical Series **September**, 6–10.

McKenzie BA, Kemp PD, Moot DJ, Mathew C, Lucas RJ (1999) Environmental effects on plant growth and development. In 'New Zealand pasture and crop science'. (Eds J White, J Hodgeson) pp. 29–44. (Oxford University Press: Auckland, New Zealand)

Metson AJ (1974) I. Some factors governing the availability of soil magnesium: a review. New Zealand journal of Experimental Agriculture **2**, 277–319. doi:10.1080/03015521.1974.10427689

Mills A, Moot DJ (2010) Annual dry matter, metabolisable energy and nitrogen yields of six dryland pastures six and seven years after establishment. In 'Proceedings of the 72nd New Zealand Grassland Association Conference', Lincoln, New Zealand. pp. 177–184. (The New Zealand Grassland Association: Dunedin, New Zealand)

Mills A, Moot DJ, Jamieson PD (2009) Quantifying the effect of nitrogen on productivity of cocksfoot (Dactylis glomerata L.) pastures. European Journal of Agronomy **30**, 63–69. doi:10.1016/j.eja.2008.07.008

Ministry for the Environment (2014) 'National policy statement for freshwater management 2014.' ME 1155. pp. 9–10. (Ministry for the Environment: Wellington, New Zealand)

Moot DJ, Brown HE, Teixeira EI, Pollock KM (2003) Crop growth and development affect seasonal priorities for lucerne management. In 'Research & practice 11: legumes for dryland pastures', Lincoln, New Zealand. pp. 201–208. (The New Zealand Grassland Association: Dunedin, New Zealand)

Nowakowski TZ (1962) Effects of nitrogen fertilizers on total nitrogen, soluble nitrogen and soluble carbohydrate contents of grass. The Journal of Agricultural Science **59**, 387–392. doi:10.1017/S0021859 600015471

Olsen SR, Cole CV, Watanabe FS (1954) 'Estimation of available phosphorus in soils by extraction with sodium bicarbonate.' (USDA: Washington, DC)

Orr RJ, Parsons AJ, Penning PD, Treacher TT (1990) Sward composition, animal performance and the potential production of grass/white clover swards continuously stocked with sheep. Grass and Forage Science **45**, 325–336. doi:10.1111/j.1365-2494.1990.tb01957.x

Rayment GE, Higginson FR (1992) 'Australian laboratory handbook of soil and water chemical methods.' (Inkata Press: Melbourne)

Sanderson MA, Labreveux M, Hall MH, Elwinger GF (2003) Nutritive value of chicory and English plantain forage. Crop Science **43**, 1797–1804. doi:10.2135/cropsci2003.1797

Stewart AV (1996) Plantain (Plantago lanceolata): a potential pasture species. Schwinning S, Parsons AJ (1996) Analysis of the coexistence mechanisms for grasses and legumes in grazing systems. Journal of Ecology **84**, 799–813.

Tamura Y, Nishibe S (2002) Changes in the concentrations of bioactive compounds in plantain leaves. Journal of Agricultural and Food Chemistry **50**, 2514–2518. doi:10.1021/jf011490x

Van Rossum MH, Bryant RH, Edwards GR (2013) Response of simple grass- white clover and multi-species pastures to gibberellic acid or nitrogen fertiliser in autumn. In 'Proceedings of the 75th New Zealand Grassland Association Conference', Tauranga, New Zealand. pp.

Totty VK, Greenwood SL, Bryant RH, Edwards GR (2013) Nitrogen partitioning and milk production of dairy cows grazing simple and diverse pastures. Journal of Dairy Science **96**, 141–149. doi:10.3168/ jds.2012-5504

145–150. (The New Zealand Grasslands Association: Dunedin, New Zealand)

VSN International (2013) 'G_{ENSTAT} for Windows.' 16th edn. (VSN International: Hemel Hempstead, UK)

Watkinson JH, Kear MJ (1994) High performance ion chromatography measurement of sulfate in 20 m_M phosphate extracts of soil. Communications in Soil Science and Plant Analysis **25**, 1015–1033. doi:10.1080/00103629409369095

Williams R (1932) Effect of a nitrogenous manure on white clover and a comparison of the productiveness of four types of white clover under simulated pasture conditions. Welsh Jour. Agrie **8**, 163–170.

Woodward SL, Waghorn GC, Bryant MA, Benton A (2012) Can diverse pasture mixtures reduce nitrogen losses. In 'Proceedings of the 5th Australasian dairy science symposium'. pp. 13–15. (Australasian Dairy Science Symposium: Melbourne)

Young DJ (1958) A study of the influence of nitrogen on the root weight and nodulation of white clover in a mixed sward. Grass and Forage Science **13**, 106–114. doi:10.1111/j.1365-2494.1958.tb00119.x

Effect of autumn regrowth interval and nitrogen fertiliser on dry matter yield and plant characteristics of six forage species

K.E. MARTIN, R.H. BRYANT, S. HODGE and G.R. EDWARDS
Faculty of Agriculture and Life Sciences, PO Box 85084,
Lincoln University. Lincoln 7647, Christchurch, New Zealand
kirsty.martin@lincolnuni.ac.nz

Abstract
Cold temperatures and drainage increase nitrogen (N) losses from livestock production systems, so autumn management and forage type were investigated as strategies to mitigate N loss whilst meeting animal requirements. The effect of regrowth interval and fertiliser rate on plant dry matter (DM) yield, plant N and digestible organic matter in the DM (DOMD) was measured in six forage species over 4 weeks regrowth, in Canterbury in autumn 2015. As regrowth interval increased, herbage DM yield increased (from 180 kg DM/ha to 922 kg DM/ha, $P<0.05$) and N response rates were highest in perennial ryegrass and plantain ($P<0.05$). Herbage N% in autumn was high at >3.2% of DM and, in grasses and herbs, was positively associated with N application rate but negatively associated with regrowth interval ($P<0.001$). Delayed grazing by up to 4 weeks, under a moderate N regime, improved herbage quality and reduced herbage N% in autumn. These results suggest plantain is a suitable alternative to perennial ryegrass to reduce N losses without impeding farm production in autumn.

Keywords: chicory, plantain, perennial ryegrass, cocksfoot, white clover, red clover, nitrate leaching, nitrogen response rate

Introduction
Reducing nitrate leaching from agricultural land is an important goal for New Zealand farmers to ensure they are within environmental regulations developed by Regional Councils across New Zealand (Ministry for the Environment 2014). These regulations will require large reductions in nitrogen (N) inputs and outputs which may affect profitability.

Nitrogen fertilisers are often used in farm systems to maintain annual herbage production, however, their excessive use is discouraged due to the impact on N loss. Martin et al. (2017) compared the effect of N fertiliser on dry matter (DM) yield and N content (N%) in a suite of forages at a set regrowth interval, and found interactions between species and N fertiliser rate. The results suggested different species may benefit from different management strategies to achieve animal production targets under a regulated N loss regime.

Nitrate leaching in pasture-based dairy systems occurs largely from urine patches due to large quantities of mineral N deposited (Di & Cameron 2002). The risk of leaching is greatest in the autumn when cooler temperatures reduce plant N uptake and higher rainfall increases drainage of soil mineral N. Plant characteristics, such as annual growth and winter activity, have been identified to improve capture of soil N (Malcolm et al. 2014; Woods et al. 2016) as well as low herbage N% to reduce urine N (Woods et al. 2016). Both annual growth and plant characteristics can be altered using farm management practises; Bryant et al. (2012) showed interactions between regrowth stage and N fertiliser on herbage N%. However, the study was restricted to perennial ryegrass and found that herbage quality declined during regrowth, reducing milk yield (Bryant et al. 2014). As little information exists on the effect of regrowth on alternate forage plant characteristics, species such as plantain, chicory, clover and other grasses, may present an opportunity to reduce N losses without impeding quality. Therefore, the objective of the study was to compare the response of different forage species to N fertiliser rate and regrowth interval with respect to their forage characteristics which mitigate N loss and maintain farm production goals.

Methods

Experimental site and design
The current study was conducted 12 months after establishment, between 10 March and 7 April 2015. The experiment was a split-split-plot design with three blocks situated under irrigation on a free-draining Templeton fine sandy loam (Immature Pallic soil, Hewitt 2010) at the Lincoln University Research Dairy Farm, Canterbury, New Zealand (43º64'S, 172º46'E). Forage species (Table 1) were the main plot treatments (area = 37.8 m2), N fertiliser rate (nil, medium and high) were the split-plot treatments (area = 6.3 m2) and regrowth interval was the split-split plot treatment (area = 0.6 m2). The N fertiliser rates were 0, 180 and 450 kg N/ha/year for grasses and herbs and 0, 156 and 389 kg N/ha/year for legumes.

Establishment and management

This study was part of a larger field experiment examining the role of N in alternative forages to reduce nitrate leaching. Details of establishment and management are described in (Martin *et al.* 2017). Briefly, the experimental area was established in March 2014 following cultivation. Control of herbage mass commenced in spring 2014 using only mower harvests, thus avoiding trampling and nutrient recycling of livestock. During the next 7 months harvest intervals in spring and summer for grasses and herbs were 32 and 26 days, respectively, and 41 and 35 days, respectively, for legumes to allow sufficient regrowth (Donaghy & Fulkerson 1998; Moot *et al.* 2003; Lee *et al.* 2015). N fertiliser was applied following each harvest as calcium ammonium nitrate (27: 0 :0 : 0; N : P :K :S), with the total annual N application rate split evenly throughout the year. Climate data was collected from Broadfields Meteorological Station, 1 km from the experimental area. Annual irrigation was 550 mm, applied between October and March. Herbage measurements Regrowth data was collected weekly between 17 March and 7 April 2015. Herbage yield, botanical composition and plant characteristics were determined by harvesting three quadrats (32 x 60 cm) per plot using hand shears, with an attachment set to 4 cm height to mimic grazing height. Harvesting occurred between 10:00 am and 12:00 pm and previously harvested areas were avoided. Herbage was kept in the shade, before being transported to the laboratory once all plots had been harvested. Two quadrats were oven-dried at 60 °C for 48 hours and weighed to determine herbage yield. The third quadrat
was mixed and a sub-sample was frozen and
freeze-dried for chemical analysis. Near-infrared spectrophotometry (NIRS, NIRSystems 5000, Foss, Maryland, USA) was used to determine plant characteristics, based on calibrations derived on experimental herbages (Martin et al. 2017). Herbage quality was measured as DOMD (digestible organic matter in dry matter) and N was measured as a % of DM. Any samples outside the calibration spectrum were analysed by wet chemistry using the same methods as Martin et al. (2017).

Statistical analysis

The split-split plot design procedure in GenStat, version 16 (VSN International 2013) was used to compare the fixed effect of forage species, N fertiliser regime, and regrowth interval on cultivars: herbage DM yield, N% and DOMD.

Results

Climate data

Average air temperature was 15.0 ºC from 10 March to 7 April 2015, this was 1.4 ºC higher than the long-term average in March/April (1981-2010). Minimum and maximum temperatures averaged between 10.7 ºC and 19.9 ºC. Rainfall was 20.8 mm from 10 March - 7 April, this was 24.7 mm below the long-term average for 1 month in March/April (1981-2010). Soil temperature (10 cm depth) was 15.5 ºC from 10 March - 7 April 2015.

Herbage DM yield

An interaction between N rate and regrowth interval ($P<0.001$) showed more rapid DM accumulation in high compared with nil N fertiliser treatments. Generally, there was a positive effect of both regrowth and N fertiliser rate on DM yield (Figure 1; Table 2). An interaction ($P<0.001$) between N fertiliser rate and species showed a positive response to N

Table 1: Forage species sown and their functional group, scientific name, cultivar and sowing rate (kg/ha).

Forage	Functional group	Scientific name	Cultivar	Sowing rate (kg/ha)
Perennial ryegrass	Grass	*Lolium perenne*	One 50 AR37	20
Cocksfoot	Grass	*Dactylis glomerata*	Savvy	8
Chicory	Herb	*Cichorium intybus*	Choice	8
Plantain	Herb	*Plantago lanceolata*	Tonic	10
White clover	Legume	*Trifolium repens*	Kopu II	5
Red clover	Legume	*Trifolium pratense*	Sensation	10

Interaction between plant species and regrowth interval showed that there were large differences between species at beginning of the regrowth interval, however, by the end of the regrowth all species were similar in DM yield apart from plantain (Figure 1; Table 2; $P=0.013$). Response to N fertiliser was highest ($P<0.05$) in plantain and perennial ryegrass (23 kg DM/kg N) compared with chicory and cocksfoot (11 kg DM/kg N).

Nutrient concentration.

The herbage N% of all treatments (sampled above 4 cm) was high, exceeding 3% of herbage DM. An interaction between N fertiliser and species revealed there was a positive relationship between N fertiliser rate and N% for herbs and grasses, but no relationship for legumes which were always had high N% (Table 2; Figure 2). Similarly, there were interactions between species and regrowth on herbage N%. Within legumes, N% did not change

with regrowth interval. In contrast, for both herbs and grasses the relationship between N% and regrowth was quadratic. During the first 2 weeks herbage N% increased then dropped by week 4 (Figure 2; Table 2; P=0.005). An interaction (P<0.001) between regrowth interval and species showed that there was no change in DOMD of red clover, while for other species, DOMD increased from week one to four (Figure 3). Differences between N fertiliser rates found DOMD were significantly higher (P<0.001) at the high N fertiliser rates, compared to the nil N fertiliser rates (Figure 3).

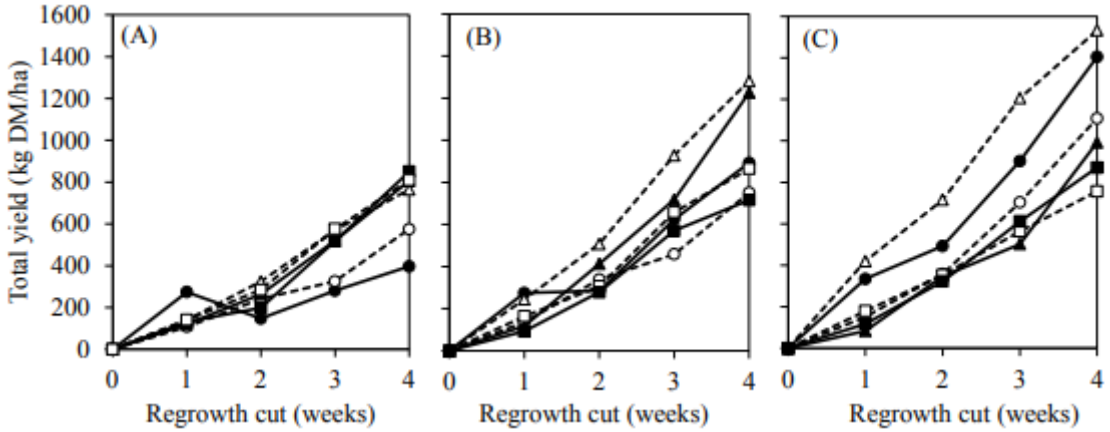

Figure 1 Effect of regrowth interval on herbage DM accumulation at three N fertiliser rates; nil (A), medium (B) and high (C), for six forage species: Perennial ryegrass (—●—), cocksfoot (- -○- -), chicory (—▲—), plantain (- -Δ- -), red clover (—■—) and white clover (- -□- -). Points are mean values.

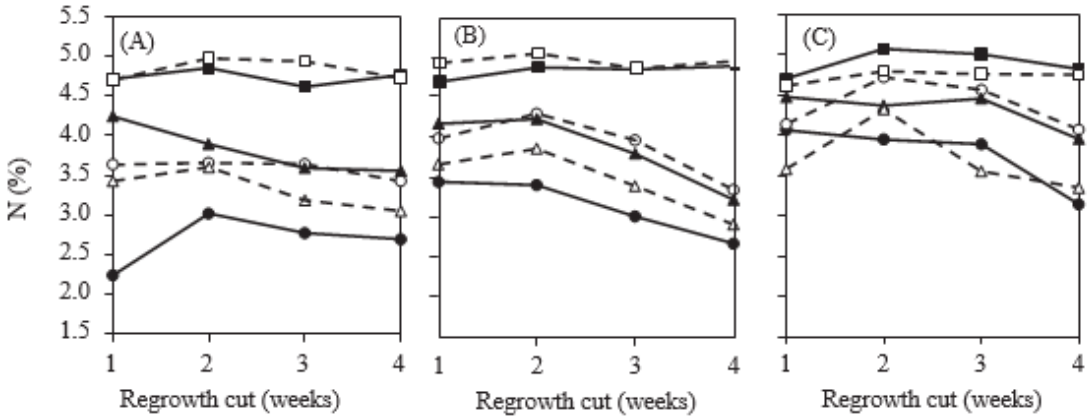

Figure 2 Effect of regrowth interval on plant N% at three N fertiliser rates; nil (A), medium (B) and high (C), for six forage species: Perennial ryegrass (—●—), cocksfoot (- -○- -), chicory (—▲—), plantain (- -Δ- -), red clover (—■—) and white clover (- -□- -). Points are mean values.

Table 2 Average DM yield, plant N% and DOMD of six forage species at three N fertiliser rates over 4 weeks. $LSD_{0.05}$= least significant difference at the 5% level. Means followed by different letters denote the values are significantly different at the 5% level.

		Yield (kg DM/ha)	N % (% of DM)	DOMD (MJ/kg DM)
Species	Chicory	509[a]	4.0[c]	77.5[c]
	Cocksfoot	438[a]	4.0[c]	70.3[a]
	Plantain	710[b]	3.5[b]	71.3[ab]
	Red clover	440[a]	4.8[d]	71.3[ab]
	Perennial ryegrass	526[a]	3.2[a]	73.1[b]
	White clover	471[a]	4.8[d]	80.0[d]
Regrowth interval (weeks)	1	180[a]	4.1[b]	70.6[a]
	2	343[b]	4.3[c]	73.8[b]
	3	624[c]	4.1[b]	75.0[c]
	4	922[d]	3.8[a]	76.9[d]
N fertiliser rate	Nil	390[a]	3.8[a]	73.1[a]
	Medium	535[b]	4.0[b]	73.8[b]
	High	626[c]	4.3[c]	75.0[b]

	P value	$LSD_{0.05}$	P value	$LSD_{0.05}$	P value	$LSD_{0.05}$
Species	**	98.3	**	0.158	**	1.755
N fertiliser rate	**	58.4	**	0.075	**	0.874
Regrowth interval	**	52.6	**	0.121	**	1.053
S x R	*	143.3	*	0.292	**	2.745
S x N	**	146.1	**	0.208	NS	2.362
R x N	**	96.8	*	0.194	NS	1.785
S x N x R	NS	239.6	NS	0.486	NS	4.487

DOMD, digestible organic matter digestibility; S, species; R, regrowth interval; N, N fertiliser rate; and NS, not significant. ** P<0.001; * P<0.01.

Discussion

Herbage DM yield

It was found that highest DM yield was achieved when N fertiliser was used on plantain and chicory at the longest regrowth interval. These findings are similar to previous studies (Minneé et al. 2013; Martin et al. 2017). On the other hand, under nil fertiliser, DM yield was greatest for legumes, so species re-ranked when N was applied. The lack of N response from legumes was expected (McKenzie et al. 1999; Martin et al. 2017) due to their N-fixing ability which indicates N was not limiting growth. Differences in growth between species, as determined by DM yield with different N rates was probably due to differences in thermal requirements (oC days) and hormonal signalling altering above and below-ground partitioning of photosynthates in response to day-length and defoliation (Moot et al. 2003; Powell et al. 2007). The autumn N response results demonstrate that perennial ryegrass and plantain need less N to produce similar DM yields compared with cocksfoot or chicory (Moore et al. 1991; King et al. 2012).Nutrient concentration The second management concern was identifying strategies to reduce herbage N content, as this is positively associated with N intake and urine N loss (Moorby 2014). At the final regrowth cut, N% was highest in legume species, intermediate in cocksfoot and chicory, and lowest in plantain and perennial ryegrass. However, herbage N contents of over 3% N (18% crude protein), which exceed cow late lactation requirements for protein, reflects the risk of high urinary N losses, irrespective of species (Castillo 2001). These results show that N% was lowest at the final harvest due to N dilution rates in the plant cells as DM increases (Blaser 1964; Peyraud & Astigarraga 1998). Similarly, moderate N fertiliser rates resulted in lower N% compared with the high N rate. Although nil N fertiliser had the lowest herbage N content, the compromise in yield is likely to offset herbage N reductions. The energy value (DOMD) of the herbage did not decline over the regrowth interval in any of the species, and remained high. This result was not expected as it was thought DOMD would generally decline (Buxton 1996; Rawnsley et al. 2002). The reason for a high DOMD throughout the regrowth was probably due to the time of year and the proportion of leaf in the herbage sampled above 4 cm. It is well documented, grasses harvested before the fourth

leaf stage are higher in DOMD due to no leaf senescence (Donaghy & Fulkerson 1998). Leaf appearance interval (time taken for one leaf to fully expand) is influenced predominantly by temperature (Silsbury 1970) and soil moisture availability (van Loo 1992). The current trial ran in autumn when cooler temperatures occurred and consequently, leaf appearance rate was slower. As a result, at week four, the grasses were at the third leaf stage and leaf senescence did not occur. These results demonstrate that delaying regrowth to 4 weeks in autumn as a management strategy to reduce N losses, does not affect herbage quality and therefore farm production goals.

Conclusions

Any farm decision to reduce N is likely to be based around whether that decision also meets socio-economic goals. As feed costs are a major component of the farm system, decisions leading to changes which reduce feed supply are likely to limit adoption. The results of this study indicate delaying grazing of grasses, herbs and legumes up to 4 weeks in autumn increases DM yield, reduces herbage N content without compromising digestibility. The strong response to N fertiliser from plantain and perennial ryegrass suggests that a moderate N fertiliser regime would address economic and environmental goals. However, because there was little management factors could do to reduce N content below 3%, future strategies to further mitigate N loss through N intake should consider low N supplements. ACKNOWLEDGEMENTS This research was undertaken as part of the Forages for Reduced Nitrate Leaching program, with principal funding form the New Zealand Ministry of Business, Innovation and Employment. The program is a partnership between DairyNZ, AgResearch and Landcare Research.

REFERENCES

Blaser, R.E. 1964. Symposium on forage utilization: effects of fertility levels and stage of maturity on forage nutritive value. 1. Journal of Animal Science 23: 246-253.

Bryant, R.H.; Dalley, D.E.; Gibbs, J.; Edwards, G.R. 2014. Effect of grazing management on herbage protein concentration, milk production and nitrogen excretion of dairy cows in mid-lactation. Grass and Forage Science 69: 644-654.

Bryant, R.H.; Gregorini, P.; Edwards, G.R. 2012. Effects of N fertilisation, leaf appearance and time of day N fractionation and chemical composition of Lolium perenne cultivars in spring. Animal Feed Science and Technology 173: 210-219.

Buxton, D.R. 1996. Quality-related characteristics of forages as influenced by plant environment and agronomic factors. Animal Feed Science and Technology 59: 37-49.

Castillo, A. 2001. Improving nitrogen utilisation in dairy cows. PhD thesis. University of Reading.

Di, H.J.; Cameron, K.C. 2002. Nitrate leaching in temperate agroecosystems: sources, factors and mitigating strategies. Nutrient Cycling in Agroecosystems 64: 237-256.

Donaghy, D.; Fulkerson, W. 1998. Priority for allocation of water-soluble carbohydrate reserves during regrowth of Lolium perenne. Grass and Forage Science 53: 211-218.

Hewitt, A.E. 2010. New Zealand soil classification. Manaaki Whenua Press, Lincoln, New Zealand.

King, C.; McEniry, J.; Richardson, M.; O'Kiely, P. 2012. Yield and chemical composition of five common grassland species in response to nitrogen fertiliser application and phenological growth stage. Acta Agriculturae Scandinavica, Section B-Soil & Plant Science 62: 644-658

Lee, J.M.; Hemmingson, N.R.; Minneé, E.M.K.; Clark, C.E.F. 2015. Management strategies for chicory (Cichorium intybus) and plantain (Plantago lanceolata): impact on dry matter yield, nutritive characteristics and plant density. Crop and Pasture Science 66: 168-183.

Malcolm, B.J.; Cameron, K.C.; Di, H.J.; Edwards, G.R.; Moir, J.L. 2014. The effect of four different pasture species compositions on nitrate leaching losses under high N loading. Soil Use and Management 30: 58-68.

Martin, K.; Edwards, G.; Bryant, R.; Hodge, M.; Chapman, D.; Moir, J.; Cameron, K. 2017. Herbage dry matter (DM) yield and nitrogen concentration of grass, legume and herb species grown at different nitrogen fertiliser rates under irrigation Animal Production Science 57: 1283-1288. https://dio.org/ 10.1071/AN16455.

McKenzie, B.A.; Kemp, P.D.; Moot, D.J.; Matthew, C.; Lucas, R.J. 1999. Environmental effects on plant growth and development. pp. 29-44. In: New Zealand pasture and crop science. Eds. White, J.; Hodgson, J. Oxford University Press: Auckland, New Zealand.

Ministry for the Environment, 2014. National policy statement for freshwater management. In: ME 1244. Ed. Ministry for the Environment.Wellington.

Minneé, E.; Clark, C.; Clark, D. 2013. Herbage production from five grazeable forages. Proceedings of the New Zealand Grassland Association 75: 245- 250.

Moorby, J. 2014. Relationship between the composition of fresh grass-based diets and the excretion of dietary nitrogen from dairy cows. The Future of European Grasslands 50: 686-689..

References

Adesogan AT, Givensz Dl, Owen E (2000) Measuring Chemical Composition and Nutritive Value in Forages. In 'Field and laboratory methods for grassland and animal production research'. (Eds L t'Manneje, RM Jones) pp. 263-275. (University Press, Cambridge: UK).

AFRC (1993) 'Energy and Protein Requirements of Ruminents.' (CAB INTERNATIONAL: Wallingford, UK).

Amrane R, Michalet-Doreau B (1993) Effect of maturity stage of Italian rye grass and lucerne on ruminal nitrogen degradability. In 'Annales de zootechnie' pp. 31-37.

Armstrong R, McCosker K, Johnson S, Millar G, Walsh K, Kuskopf B, Probert M, Standley J (1999) Legume and opportunity cropping systems in central Queensland. 1. Legume growth, nitrogen fixation, and water use. *Crop and Pasture Science* **50**, 909-924.

Bach A, Calsamiglia S, Stern MD (2005) Nitrogen Metabolism in the Rumen. *Journal of Dairy Science* **88**, E9-E21.

Ball CC, Parsons AJ, Rasmussen S, Shaw C, Rowarth JS (2012) Seasonal differences in the capacity of perennial ryegrass to respond to gibberellin explained. *Proceedings of the New Zealand Grassland Association* **74**, 183-188.

Ball PR, Field TRO (1982) Responses to nitrogen as affected by pasture characteristics, season and grazing management. In 'Nitrogen fertilisers in New Zealand Agriculture, New Zealand Institure of Agricultural Science.'. (Ed. PB Lynch) pp. 45-64. (Chapter 4: Wellington).

Barker D, Lancashire J, Moloney S, Dymock N, Stevens D, Turner J, Scott D, Archie W (1993) Introduction, production, and persistence of five grass species in dry hill country: 8. Summary and conclusions. *New Zealand Journal of Agricultural Research* **36**, 61-66.

Barry T (1998) The feeding value of chicory (Cichorium intybus) for ruminant livestock. *The Journal of Agricultural Science* **131**, 251-257.

Beever DE, Losada HR, Cammell CB, Evans R, T., Haines MJ (1986) Effect of forage species and season on nutrient digestion and supply in grazing cattle. *British Journal of Nutrition* **56**, 209-255.

Belesky DP, Turner KE, Ruckle JM (2000) Influence of Nitrogen on Productivity and Nutritive Value of Forage Chicory. *Agronomy Journal* **92**, 472-478.

Belton J (1992) The Evaluation of Matua Prairie Grass for Use on Dairy Farms in Tasmania. Final Report. *Daily Research and Development Corporation, Hobart, Tas., Australia*.

Berendse F (1982) Competition between plant populations with different rooting depths. *Oecologia* **53**, 50-55.

Bergström L, Johansson R (1991) Leaching of nitrate from monolith lysimeters of different types of agricultural soils. *Journal of Environmental quality* **20**, 801-807.

Beukes PC, Gregorini P*, et al.* (2014) The potential of diverse pastures to reduce nitrogen leaching on New Zealand dairy farms. *Animal Production Science* **54**, 1971-1979.

Bittman S, Kowalenko C (2000) Within-season grass herbage crude protein and nitrate N concentrations as affected by rates and seasonal distribution of fertilizer nitrogen in a high yearly rainfall climate. *Canadian Journal of Plant Science* **80**, 277-285.

Blaser RE (1964) Symposium on forage utilization: effects of fertility levels and stage of maturity on forage nutritive value. *Journal of animal science* **23**, 246-253.

Box L, Edwards GR, Bryant RH (2016) Milk production and urinary nitrogen excretion of dairy cows grazing perennial ryegrass-white clover and pure plantain swards. *Proceedings of the New Zealand Society of Animal Production* **76**, 18-21.

Box LA, Edwards GR, Bryant RH (2017) Milk production and urinary nitrogen excretion of dairy cows grazing plantain in early and late lactation. *New Zealand Journal of Agricultural Research* **60**, 470-482.

Bremner J (1965) Total nitrogen. *Methods of soil analysis. Part 2. Chemical and microbiological properties*, 1149-1178.

Brock J (1973) Growth and nitrogen fixation of pure stands of three pasture legumes with high/low phosphate. *New Zealand Journal of Agricultural Research* **16**, 483-491.

Brookes IM, Nicol AM (2007) Chapter 11: The protein requirements of grazing livestock. In 'Pasture Supplements for Grazing Animals' p. 173. (Hamilton, N.Z. : New Zealand Society of Animal Production.

Brown HE, Moot DJ, Pollock KM (2005) Herbage production, persistence, nutritive characteristics and water use of perennial forages grown over 6 years on a Wakanui silt loam. *New Zealand Journal of Agricultural Research* **48**, 423-439.

Brown W, Pitman W (1991) Concentration and degradation of nitrogen and fibre fractions in selected tropical grasses and legumes. *Tropical Grasslands* **25**, 305-312.

Bryant J, Hoogendoorn C, Snow V (2007) Simulation of mitigation strategies to reduce nitrogen leaching from grazed pasture. *Proceeding of the New Zealand Grassland Association* **69**, 145.

Bryant RH, Dalley DE, Gibbs J, Edwards GR (2014) Effect of grazing management on herbage protein concentration, milk production and nitrogen excretion of dairy cows in mid-lactation. *Grass and Forage Science* **69**, 644-654.

Bryant RH, Gregorini P, Edwards GR (2012) Effects of N fertilisation, leaf appearance and time of day N fractionation and chemical composition of *lolium perenne* cultivars in spring. *Animal Feed Science and Technology* **173**, 210-219.

Burke J, Waghorn G, Brookes I, Attwood G, Kolver E (2000) Formulating total mixed rations from forages-defining the digestion kinetics of contrasting species. In 'Proceedings of the New Zealand Society of Animal Production' pp. 9-14.

Buxton DR (1996) Quality-related characteristics of forages as influenced by plant environment and agronomic factors. *Animal Feed Science and Technology* **59**, 37-49.

Cameron K, Di H, Moir J, Christie R, Pellow R (June 2005) Using nitrogen: what is best practice? In 'South Island Dairy Event (SIDE) Proceedings, Lincoln University, June 2005'.

Cameron K, Di HJ, Moir JL (2013) Nitrogen losses for the soil/plant system: a review. *Annals of Applied Biology* **162**, 145-173.

Cameron KC (1992) Nitrogen in Soil. *Encyclopedia of Earth System Science* **3**, 307-317.

Caradus J, Woodfield D, Stewart A (1996) Overview and vision for white clover. *Special Publication - Agronomy Society of New Zealand*, 1-6.

Carey P, Cameron K, Di H, Edwards G (2017) Comparison of nitrate leaching from oats and Italian ryegrass catch crops following simulated winter forage grazing: a field lysimeter study. *New Zealand Journal of Agricultural Research* **60**, 298-318.

Carey P, Cameron K, Di H, Edwards G, Chapman D (2016) Sowing a winter catch crop can reduce nitrate leaching losses from winter-applied urine under simulated forage grazing: a lysimeter study. *Soil Use and Management* **32**, 329-337.

Cassman KG, Dobermann A, Walters DT (2002) Agroecosystems, nitrogen-use efficiency and nitrogen management. *AMBIO: A Journal of the Human Environment* **31**, 132-140.

Castillo A (2001) PhD Thesis - Improving nitrogen utilisation in dairy cows. University of Reading.

Castillo AR, Kebreab E, Beever DE, Barbi JH, Sutton JD, Kirby HC, France J (2001) The effect of protein supplementation on nitrogen utilization in lactating dairy cows fed grass silage diets. *Journal of animal science* **79**, 247-253.

Chapman D, Edwards G*, et al.* (2017a) Nitrogen leaching, production and profit of irrigated dairy systems using either low or high inputs of fertiliser and feed: The Pastoral 21 experience in Canterbury. *Science and policy: nutrient management challenges for the next generation. (Eds LD Currie and MJ Hedley). http://flrc. massey. ac. nz/publications. html. Occasional Report*.

Chapman DF, Edwards GR, Pinxterhuis JB (2014) Plants for dairy grazing systems operating under nitrate leaching limits. *Proceedings of the New Zealand Society of Animal Production* **74**, 102-107.

Chapman DF, Lee JM, Rossi L, Edwards GR, Pinxterhuis JB, Minnee EMK (2017b) White clover: the forgotten component of high-producing pastures? *Animal Production Science* **57**, 1269-1276.

Cheng L, Judson HG, Bryant RH, Mowat H, Guinot L, Hague H, Taylor S, Edwards GR (2017) The effects of feeding cut plantain and perennial ryegrass-white clover pasture on dairy heifer feed and water intake, apparent nutrient digestibility and nitrogen excretion in urine. *Animal Feed Science and Technology* **229**, 43-46.

Christensen C, Hedley M, Hanly J, Horne D (2012a) Nitrogen loss mitigation using duration-controlled grazing: Field observations compared to modelled outputs. *Proceedings of the New Zealand Grassland Association* **74**, 115-120.

Christensen C, Hedley M, Hanly J, Horne D (2012b) Three years of durationcontrolled grazing: what have we found. *Advanced nutrient management: Gains from the past–goals for the future. (Eds LD Currie and MJ Hedley). http://flrc. massey. ac. nz/publications. html. Occasional Report*.

Chrystal J, Monaghan R, Hedley M, Horne D (2016) Design of a low cost winter stand-off pad for reducing nutrient losses to water from winter forage crops grazed by dairy cows. *Integrated nutrient and water management for sustainable farming. . (Eds LD Currie and MJ Hedley). http://flrc. massey. ac. nz/publications. html. Occasional Report*.

Ciavarella T, Simpson R, Dove H, Leury B, Sims I (2000) Diurnal changes in the concentration of water-soluble carbohydrates in Phalaris aquatica L. pasture in spring, and the effect of short-term shading. *Australian Journal of Agricultural Research* **51**, 749-756.

Clark DA, Anderson CB, Berquist T (1990) Growth rates of 'Grasslands Puna' chicory (Cichorium intybus L.) at various cutting intervals and heights and rates of nitrogen. *New Zealand Journal of Agricultural Research* **33**, 213-217.

Clark DA, Penno JW, Neil PG (1996) Nutritional merits and problems of pasture. In 'Milk composition, production and biotechnology'. (Eds RAS Welch, DWJ Burns, SR Davis, AI Popay, CG Prosser) pp. 397-418. (CAB International: Wallingford, UK).

Clement AR, Dalley DE, Chapman DF, Edwards GR, Bryant RH (2016) Effect of grazing system on nitrogen partitioning in lactating dairy cows grazing irrigated pastures in Canterbury, New Zealand. *Proceedings of the New Zealand Society of Animal Production* **76**, 94-99.

Coblentz W, Akins M, Cavadini J, Jokela W (2017) Net effects of nitrogen fertilization on the nutritive value and digestibility of oat forages. *Journal of Dairy Science* **100**, 1739-1750.

Collins M, McCoy JE (1997) Chicory productivity, forage quality, and response to nitrogen fertilization. *Agronomy Journal* **89**, 232-238.

Cosgrove G, Burke J, Death A, Hickey M, Pacheco D, Lane G (2007) Ryegrasses with increased water soluble carbohydrate: evaluating the potential for grazing dairy cows in New Zealand. *Proceedings of the New Zealand Grasslands Association* **69**, 179.

Cosgrove G, Koolaard J, Luo D, Burke J, Pacheco D (2009) The composition of high sugar ryegrasses. *Proceedings of the New Zealand Grassland Association* **71**, 187-193.

Cowling D (1961) The effect of nitrogenous fertilizer on an established white clover sward. *Grass and Forage Science* **16**, 65-68.

Da Silva MS, Tremblay GF, Bélanger G, Lajeunesse J, Papadopoulos YA, Fillmore SA, Jobim CC (2014) Forage energy to protein ratio of several legume–grass complex mixtures. *Animal Feed Science and Technology* **188**, 17-27.

Dairy NZ (2017) Pasture based production systems. In 'Facts and Figures for NZ Dairy Farmers '. (Ed. Dairy NZ). (Dairy NZ: Hamilton, NZ).

Dale T, Greenfield R, Tozer K, Cameron C (2017) Post-sampling procedure affects the measured nutritive value of perennial ryegrass (Lolium perenne). *Journal of New Zealand Grasslands* **79**, 257-264.

De Klein CAM, Ledgard SF (2001) An analysis of environmental and economic implications of nil and restricted grazing systems designed to reduce nitrate leaching from New Zealand dairy farms. I. Nitrogen losses. *New Zealand Journal of Agricultural Research* **44**, 201-215.

Deaker J, Young M, Fraser T, Rowarth J (1994) Carcass, liver and kidney characteristics of lambs grazing plantain (Plantago lanceolata), chicory (Chichorium intybus), white clover (Trifolium repens) or perennial ryegrass (Lolium perenne). In 'New Zealand Society of Animal Production ' pp. 197-197. (New Zealand Society of Animal Production).

Di H, Cameron K (2000) Calculating nitrogen leaching losses and critical nitrogen application rates in dairy pasture systems using a semi-empirical model. *New Zealand Journal of Agricultural Research* **43**, 139-147.

Di H, Cameron K (2002a) Nitrate leaching in temperate agroecosystems: sources, factors and mitigating strategies. *Nutrient Cycling in Agroecosystems* **64**, 237-256.

Di H, Cameron K (2002b) The use of a nitrification inhibitor, dicyandiamide (DCD), to decrease nitrate leaching and nitrous oxide emissions in a simulated grazed and irrigated grassland. *Soil Use and Management* **18**, 395-403.

Di HJ, Cameron K, Shen JP, Winefield CS, O'Callaghen M, Bowatee S, GHe JZ (2010) Ammonia-oxidising bacteria and archaea grow under contrasting soil nitrogen conditions. *FEMS Microbiology Ecology* **72**, 386-394.

Di HJ, Cameron KC (2002c) Nitrate leaching in temperate agroecosystems: sources, factors and mitigating strategies. *Nutrient Cycling in Agroecosystems* **64**, 237-256.

Dietz M, Machill S, Hoffmann HC, Schmidtke K (2013) Inhibitory effects of Plantago lanceolata L. on soil N mineralization. *Plant and Soil* **368**, 445-458.

Donaghy D, Fulkerson W (1998) Priority for allocation of water-soluble carbohydrate reserves during regrowth of Lolium perenne. *Grass and Forage Science* **53**, 211-218.

Edwards G, Parsons A, Rasmussen S, Bryant R (2007) High sugar ryegrasses for livestock systems in New Zealand. *Proceeding of the New Zealand Grassland Association* **69**, 161-171.

Elgersma A, Hassink J (1997) Effects of white clover (Trifolium repens L.) on plant and soil nitrogen and soil organic matter in mixtures with perennial ryegrass (Lolium perenne L.). *Plant and Soil* **197**, 177-186.

Elizalde JC, Merchen NR, Faulkner DB (1999) Fractionation of fiber and crude protein in fresh forages during the spring growth. *Journal of animal science* **77**, 476-484.

Fertresearch (2011) Code of Practice for Nutrient Management: Nitrification Inhibitors - Fact sheet 11
https://secure.overseer.org.nz/test/Content/Help/Content/Code_of_practice_Fact_Sheets_All.pdf.

Fraser T (1982) Evaluation of 'Grasslands Matua'prairie grass and 'Grasslands Maru'phalaris with and without lucerne in Canterbury. *New Zealand journal of Experimental Agriculture* **10**, 235-237.

Fraser TJ, Rowarth JS (1996) Legumes, herbs or grass for lamb performance? *Proceedings of the New Zealand Grassland Association* **58**, 49-52.

Fulkerson W, Horadagoda A, Neal J, Barchia I, Nandra K (2008) Nutritive value of forage species grown in the warm temperate climate of Australia for dairy cows: Herbs and grain crops. *Livestock Science* **114**, 75-83.

Fulkerson WJ, Donaghy DJ (2001) Plant-soluble carbohydrate reserves and senescence - key criteria for developing an effective grazing management system for ryegrass-based pastures: a review. *Australian Journal of Experimental Agriculture* **41**, 261-275.

Fulkerson WJ, Fennell JFM, Slack K (2000) Production and forage quality of prairie grass in comparison to perennial ryegrass and tall fescue in subtropical dairy pastures. *Australian Journal of Experimental Agriculture* **40**, 1059-1068.

Fulkerson WJ, Neal JS, Clark CF, Horadagoda A, Nandra KS, Barchia I (2007) Nutritive value of forage species grown in the warm temperate climate of Australia for dairy cows: Grasses and legumes. *Livestock Science* **107**, 253-264.

Fulkerson WJ, Slack K (1994) Leaf number as a criterion for determining defoliation time for Lolium perenne, 1. Effect of water-soluble carbohydrates and senescence. *Grass and Forage Science* **49**, 373-377.

Garwood E, Ryden J (1986) Nitrate loss through leaching and surface runoff from grassland: effects of water supply, soil type and management. *Nitrogen fluxes in intensive grassland systems*, 99-113.

Getachew G, Crovetto G, Fondevila M, Krishnamoorthy U, Singh B, Spanghero M, Steingass H, Robinson P, Kailas M (2002) Laboratory variation of 24 h in vitro gas production and estimated metabolizable energy values of ruminant feeds. *Animal Feed Science and Technology* **102**, 169-180.

Goh KM, Bruce GE (2005) Comparison of biomass production and biological nitrogen fixation of multi-species pastures (mixed herb leys) with perennial ryegrass-white clover pasture with and without irrigation in Canterbury, New Zealand. *Agriculture, Ecosystems and Environment* **110**, 230-240.

Goswami A, Willcox J (1969) Effect of applying increasing levels of nitrogen to ryegrass: I.—Composition of various nitrogenous fractions and free amino acids. *Journal of the Science of Food and Agriculture* **20**, 592-595.

Grant CA, Flaten DN, Tomasiewicz DJ, Sheppard SC (2001) The importance of early season phosphorus nutrition. *Canadian Journal of Plant Science* **81**, 211-224.

Hannaway D, Shuler P (1993) Nitrogen fertilization in alfalfa production. *Journal of Production Agriculture* **6**, 80-85.

Harris PB, McNaught KJ, Lynch PB (1966) The main effects and interactions of nutrients on the growth of irrigated lucerne. *New Zealand Journal of Agricultural Research* **9**, 653-690.

Harris S, Clark D, Auldist M, Waugh C, Laboyrie P (1997) Optimum white clover content for dairy pastures. *Proceedings of the New Zealand Grassland Association* **59**, 29-33.

Hayes RC, Dear BS, Li GD, Virgona JM, Conyers MK, Hackney BF, Tidd J (2010) Perennial pastures for recharge control in temperate drought-prone environments. Part 1: productivity, persistence and herbage quality of key species. *New Zealand Journal of Agricultural Research* **53**, 283-302.

Haynes RJ, Williams P (1993) Nutrient cycling and soil fertility in the grazed pasture ecosystem *Advances in Agronomy* **49**, 119-199.

Hewitt AE (2010) 'New Zealand Soil Classification ' (Manaaki Whenua Press: Lincoln, NZ).

Hill JO, Simpson RJ, Wood JT, Moore AD, Chapman DF (2005) The phosphorus and nitrogen requirements of temperate pasture species and their influence on grassland botanical composition. *Australian Journal of Agricultural Research* **56**, 1027-1039.

Hill MJ, Hampton JG, Rowarth JS (2007) Herbage seeds. In 'New Zealand Pasture and Crop Science'. (Eds J White, J Hodgson) pp. 249-268. (Oxford University Press: Auckland).

Hoekstra NJ, Struik PC, Lantinga EA, Schulte RPO (2007) Chemical composition of lamina and sheath of Lolium perenne as affected by herbage management. *NJAS - Wageningen Journal of Life Sciences* **55**, 55-73.

Hoekstra NJ, Struik PC, Lantinga EA, Van Amburgh ME, Schulte RPO (2008) Can herbage nitrogen fractionation in Lolium perenne be improved by herbage management? *NJAS - Wageningen Journal of Life Sciences* **55**, 167-180.

Hoglund J, Dougherty C, Langer R (1974) Response of irrigated lucerne to defoliation and nitrogen fertiliser. *New Zealand journal of Experimental Agriculture* **2**, 7-11.

Holmes CW, Brookes IM, Garrick DJ, Mackenzie DDS, Parkingson TJ, Wilson GF (2007a) Feeding the herd: Feed requirements and feeding levels. In 'Milk Production from Pasture - Priciples and Practices'. (Ed. D Swain) pp. 53-68. (Massey University: Palmerston North).

Holmes CW, Brookes IM, Garrick DJ, MacKenzie DDS, Parkinson TJ, Wilson GF (2007b) Nutrition: Classification and Utilisation of Nutrients. In 'Milk Production from Pasture: Principles and Practices'. (Ed. D Swain) pp. 209-234. (Massey University,: Palmerston North).

Hörtensteiner S, Feller U (2002) Nitrogen metabolism and remobilization during senescence. *Journal of Experimental Botany* **53**, 927-937.

Hume D (1991) Leaf and tiller production of prairie grass (Bromus willdenowii Kunth) and two ryegrass (Lolium) species. *Annals of Botany* **67**, 111-121.

Hunt W, Field T (1979) Growth characteristics of perennial ryegrass. *Proceedings of the New Zealand Grassland Association* **40**, 104-113.

Huntington GB (1984) Net absorption of glucose and nitrogenous compounds by lactating Holstein cows. *Journal of Dairy Science* **67**, 1919–1927.

Jarvis SC, Stockdale EA, Shepherd MA, Powlson DS (1996) Nitrogen mineralization in temperate agricultural soils: processes and measurement. *Advances in Agronomy* **57**, 187-235.

Johnson C, Reiling B, Mislevy P, Hall M (2001) Effects of nitrogen fertilization and harvest date on yield, digestibility, fiber, and protein fractions of tropical grasses. *Journal of Animal Science* **79**, 2439-2448.

Kebreab E, France J, Beever DE, Castillo AR (2001) Nitrogen pollution by dairy cows and its mitigation by dietary manipulation. *Nutrient Cycling in Agroecosystems* **60**, 275-285.

Keeney D, Bremner J (1966) Comparison and evaluation of laboratory methods of obtaining an index of soil nitrogen availability. *Agronomy Journal* **58**, 498-503.

Kemp PD, Condron LM, Mathew C (1999a) Pastures and Soil Fertility. In 'New Zealand Pasture and Crop Science'. (Eds J White, J Hodgson) pp. 67-82. (Oxford University Press: Auckland).

Kemp PD, Mathew C, Lucas RJ (1999b) Pasture species and cultivars. In 'New Zealand Pasture and Crop Science'. (Eds J White, J Hodgson) pp. 83-99. (Oxford University Press: Auckland).

King C, McEniry J, Richardson M, O'Kiely P (2012) Yield and chemical composition of five common grassland species in response to nitrogen fertiliser application and phenological growth stage. *Acta Agriculturae Scandinavica, Section B–Soil & Plant Science* **62**, 644-658.

Kingston - Smith AH, Theodorou MK (2000) Post-ingestion metabolism of fresh forage. *New Phytologist* **148**, 37–55.

Kirchhof S, Eisner I, Gierus M, Südekum KH (2010) Variation in the contents of crude protein fractions of different forage legumes during the spring growth. *Grass and Forage Science* **65**, 376-382.

Labreveux M, Hall MH, Sanderson M (2004) Productivity of chicory and plantain cultivars under grazing. *Agronomy Journal* **96**, 710-716.

Lancashire J, Brock J (1983) Management of new cultivars for dryland. *Proceedings of the New Zealand Grassland Association* **44**, 61-73.

Lapierre H, Berthiaume R, Raggio G, Thivierge M, Doepel L, Pacheco D, Dubreuil P, Lobley G (2005) The route of absorbed nitrogen into milk protein. *Animal Science* **80**, 11-22.

Ledgard S, Law G, Dragten R, Power I, Webby R, Dooley E (2006a) Benchmarking of farms around Lake Taupo and alternative farm system options: issues and implications. . In 'Implementing Sustainable Nutrient Management Strategies in Agriculture.'. (Ed. HJ Currie LD) pp. 445-451. (Occasional Report No. 19. : Research Centre, Massey University, Palmerston North).

Ledgard S, O'Connor M, Carruthers V, Brier G (1990) Variability in pasture nitrogen fractions and the relationship to bloat. *New Zealand Journal of Agricultural Research* **33**, 237-242.

Ledgard S, Schils R, Eriksen J, Luo J (2009) Environmental impacts of grazed clover/grass pastures. *Irish Journal of Agricultural and Food Research*, 209-226.

Ledgard S, Sprosen M, Judge A, Lindsey S, Jensen R, Clark D, Luo J (2006b) Nitrogen leaching as affected by dairy intensification and mitigation practices in the resource efficient dairying (RED) trial. *Implementing sustainable nutrient management strategies in agriculture. (Eds LD Currie and MJ Hedley). http://flrc. massey. ac. nz/publications. html. Occasional Report.*

Ledgard SF (2001) Nitrogen cycling in low input legume-based agriculture, with emphasis on legume/grass pastures. *Plant and Soil* **228**, 43-59.

Lee JM, Hemmingson NR, Minnee EMK, Clark CEF (2015) Management strategies for chicory (Cichorium intybus) and plantain (Plantago lanceolata): impact on dry matter yield, nutritive characteristics and plant density. *Crop and Pasture Science* **66**, 168-183.

Li FY, Betteridge K, Cichota R, Hoogendoorn CJ, Jolly BH (2012) Effects of nitrogen load variation in animal urination events on nitrogen leaching from grazed pasture. *Agriculture, ecosystems & environment* **159**, 81-89.

Li G, Kemp P, Hodgson J (1997) Biomass allocation, regrowth and root carbohydrate reserves of chicory (Cichorium intybus) in response to defoliation in glasshouse conditions. *The Journal of Agricultural Science* **129**, 447-458.

Licitra G, Hernandez TM, Van Soest PJ (1996) Standardization of procedures for nitrogen fractionation of ruminant feeds. *Animal Feed Science and Technology* **57**, 347-358.

Loaiza PA, Balocchi O, Bertrand A (2016) Carbohydrate and crude protein fractions in perennial ryegrass as affected by defoliation frequency and nitrogen application rate. *Grass and Forage Science*.

Malcolm BJ, Cameron KC, Di HJ, Edwards GR, Moir JL (2014) The effect of four different pasture species compositions on nitrate leaching losses under high N loading. *Soil Use and Management* **30**, 58-68.

McKenzie BA, Kemp PD, Moot DJ, Matthew C, Lucas RJ (1999) Environmental effects on plant growth and development. In 'New Zealand Pasture and Crop Science'. (Eds J White, J Hodgson) pp. 29-44. (Oxford University Press: Auckland, New Zealand).

McLaren RG, Cameron KC (1996a) Classification and Characteristics of New Zealand Soils. In 'Soil Science - Sustainable Production and Environmental Protection'. (Eds RG McLaren, KC Cameron) pp. 28-42. (Oxford University Press: Melborne).

McLaren RG, Cameron KC (1996b) Soil, plant and fertiliser nitrogen In 'Soil Science - Sustainable Production and Envrionmental Protection'. (Eds RG McLaren, KC Cameron) pp. 192-207. (Oxford Univercity Press: Melborne).

McLaren RG, Cameron KC (1996c) Water and Solute Movement in Soils and Plants. In 'Soil Science - Sustainable Production and Envrionmental Protection'. (Eds RG McLaren, KC Cameron) pp. 87-104. (Oxford Univercity Press: Melborne).

Metson AJ (1974) I. Some factors governing the availability of soil magnesium: A review. *New Zealand journal of Experimental Agriculture* **2**, 277-319.

Miller LA, Moorby JM, Davies DR, Humpherys MO, Scollan ND, MacRae JC, Theodorou MK (2001) Increased concentration of water-soluble carbohydrate in perennial ryegrass (*Lolium perenne L.*): milk production from late-lactation dairy cows. *Grass and Forage Science* **56**, 383-394.

Mills A, Moot DJ (2010) Annual dry matter, metabolisable energy and nitrogen yields of six dryland pastures six and seven years after establishment. *Proceedings of the New Zealand Grassland Association* **72**, 177-184.

Mills A, Moot DJ, Jamieson PD (2009) Quantifying the effect of nitrogen on productivity of cocksfoot (Dactylis glomerata L.) pastures. *European Journal of Agronomy* **30**, 63-69.

Ministry for the Environment (2014) National Policy Statement for Freshwater Management *Ed. Ministry for the Environment (Wellington)* http://www.mfe.govt.nz/publications/fresh-water/national-policy-statement-freshwater-management-2014-amended-2017.

Minneé E, Clark C, Clark D (2013) Herbage production from five grazeable forages. *Proceedings of the New Zealand Grassland Association* **75**, 245-250.

Moir J, Cameron K, Di H (2007) Effects of the nitrification inhibitor dicyandiamide on soil mineral N, pasture yield, nutrient uptake and pasture quality in a grazed pasture system. *Soil Use and Management* **23**, 111-120.

Moir JL, Cameron KC, Di HJ, Fertsak U (2011) The spatial coverage of dairy cattle urine patches in an intensively grazed pasture system. *The Journal of Agricultural Science* **149**, 473-485.

Moir JL, Cameron KC, Di HJ, Roberts AH, Kuperus W (2003) The effects of urea and ammonium sulphate nitrate (ASN) on the production and quality of irrigated dairy pastures in Canterbury, New

Zealand. In 'Tools for nutrient and pollutant management: Applications to agriculture and environmental quality'. Massey University, Palmerston North, New Zealand. (Eds LD Currie, JA Hanly) pp. 139-145. (Fertilizer and Lime Research Centre, Massey University).

Moir JL, Edwards GR, Berry LN (2013) Nitrogen uptake and leaching loss of thirteen temperate grass species under high N loading. *Grass and Forage Science* **68**, 313-325.

Monaghan R, Hedley M, Di H, McDowell R, Cameron K, Ledgard S (2007) Nutrient management in New Zealand pastures—recent developments and future issues. *New Zealand Journal of Agricultural Research* **50**, 181-201.

Monaghan R, Paton R, Smith L, Drewry J, Littlejohn R (2005) The impacts of nitrogen fertilisation and increased stocking rate on pasture yield, soil physical condition and nutrient losses in drainage from a cattle-grazed pasture. *New Zealand Journal of Agricultural Research* **48**, 227-240.

Moorby JM (2014) Relationship between the composition of fresh grass-based diets and the excretion of dietary nitrogen from dairy cows. *EGF at 50: The future of European grasslands. Proceedings of the 25th General Meeting of the European Grassland Federation, Aberystwyth, Wales, 7-11 September 2014.* **50**, 686-689.

Moorby JM, Evans RT, Scollan ND, MacRae JC, Theodorou MK (2006) Increased concentration of water-soluble carbohydrate in perennial ryegrass (Lolium perenne L.). Evaluation in dairy cows in early lactation. *Grass and Forage Science* **61**, 52-59.

Moore K, Moser LE, Vogel KP, Waller SS, Johnson B, Pedersen JF (1991) Describing and quantifying growth stages of perennial forage grasses. *Agronomy Journal* **83**, 1073-1077.

Moot DJ, Brown HE, Teixeira EI, Pollock KM (2003) Crop growth and development affect seasonal priorities for lucerne management. In 'Legumes for Dryland Pastures'. Lincoln University pp. 18-19. (Proceedings of a New Zealand Grassland Association Inc Symposium).

Moot DJ, Scott WR, Roy AM, Nicholls AC (2000) Base Temperature and Thermal Time Requirements for Germination and Emergenceof Temerate Pasture Species. *New Zealand Journal of Agricultual Research* **43**, 15-25.

Navarrete S, Kemp PD, Pain SJ, Back PJ (2016) Bioactive compounds, aucubin and acteoside, in plantain (Plantago lanceolata L.) and their effect on in vitro rumen fermentation. *Animal Feed Science and Technology* **222**, 158-167.

Nevens F, Rehuel D (2003) Effects of cutting or grazing grass swards on herbage yield, nitrogen uptake and residual soil nitrate at different levels of N fertilization. *Grass and Forage Science* **58**, 431-449.

Nobilly F, Bryant R, McKenzie B, Edwards G (2013) Productivity of rotationally grazed simple and diverse pasture mixtures under irrigation in Canterbury. *Proceedings of the New Zealand Grassland Association* **75**, 165-172.

Nowakowski T, Byers M (1972) Effects of nitrogen and potassium fertilisers on contents of carbohydrates and free amino acids in Italian ryegrass. II. Changes in the composition of the non-protein nitrogen fraction and the distribution of individual amino acids. *Journal of the Science of Food and Agriculture* **23**, 1313-1333.

O'Connor M (1982) Nitrogen fertilisers for the production of out-of-season grass. *Nitrogen fertilisers in New Zealand Agriculture"(PB Lynch, ed.)*, 65-76.

O'connell C, Judson H, Barrell G (2016) Sustained diuretic effect of plantain when ingested by sheep. *Proceedings of the New Zealand Society of Animal Production* **76**, 14-17.

O'Connor KF (1990) Chapter 5: Pasture and Soil Fertility. In 'Pastures: Their Ecology and Management'. (Ed. RHM Langer) pp. 157-196. (Oxford University Press: Auckland).

Olsen SR, Cole CV, Watanabe FS (1954) 'Estimation of available phosphorus in soils by extraction with sodium bicarbonate. Circular / United States Department of Agriculture; no. 939.' (USDA: Washington).

Orr RJ, Parsons AJ, Penning PD, Treacher TT (1990) Sward composition, animal performance and the potential production of grass/white clover swards continuously stocked with sheep. *Grass and Forage Science* **45**, 325-336.

Pacheco D, Waghorn G (2008) Dietary nitrogen–definitions, digestion, excretion and consequences of excess for grazing ruminants. *Proceedings of the New Zealand Grassland Association* **70**, 107-116.

Parsons A, Rasmussen S, Xue H, Newman J, Anderson C, Cosgrove G (2004) Some 'high sugar grasses' don't like it hot. *Proceedings of the New Zealand Grassland Association* **66**, 265-271.

Parsons AJ, Edwards GR, Newton PCDC, D.F, Caradus JR, Rasmussen S, Rowarth JS (2011) Past lessons and future prospects: plant breeding for yield and persitance in cool-temperate pastures. *Grass and Forage Science* **66**, 153-172.

Peyraud JL, Astigarraga L (1998) Review of the effect of nitrogen fertilization on the chemical composition, intake, digestion and nutritive value of fresh herbage: consequences on animal nutrition and N balance. *Animal Feed Science and Technology* **72**, 235-259.

Pichard G, Van Soest PJ (1977) Protein solubility of ruminant feeds. *Proc. Cornell Nutr. Conf., Department of Animal Science, Cornell University, Ithaca, NY*, 91–98.

Pincay-Figueroa P, Lopez-Gonzalez F, Velarde-Guillen J, Heredia-Nava D, Martinez-Castaneda F, Vicente F, Martinez-Fernandez A, Arriaga-Jordan C (2016) Cut and carry vs. grazing of cultivated pastures in small-scale dairy systems in the central highlands of Mexico. *Journal of Agriculture and Environment for International Development (JAEID)* **110**, 349-363.

Powell AM, Kemp PD, Jaya I, D., Osoborne MA (2007) Establishment, growth and development of plantain and chicory under grazing. *Proceedings of the New Zealand Grassland Association* **69**, 41-45.

Raab L, Cafantaris B, Jilg T, Menke K (1983) Rumen protein degradation and biosynthesis: 1. A new method for determination of protein degradation in rumen fluid in vitro. *British Journal of Nutrition* **50**, 569-582.

Rasmussen S, Parsons A, Xue H, Newman J (2009) High sugar grasses–harnessing the benefits of new cultivars through growth management. *Proceedings of the New Zealand Grassland Association* **71**, 167-175.

Rawnsley RP, Donaghy DJ, Fulkerson WJ, Lane PA (2002) Changes in the physiology and feed quality of cocksfoot (Dactylis glomerata L.) during regrowth. *Grass and Forage Science* **57**, 203-211.

Rayment GE, Higginson FR (1992) 'Australian laboratory handbook of soil and water chemical methods.' (Inkata Press Pty Ltd: Melbourne, Australia).

Roche J, Ledgard S, Sprosen M, Lindsey S, Penno J, Horan B, Macdonald K (2016) Increased stocking rate and associated strategic dry-off decision rules reduced the amount of nitrate-N leached under grazing. *Journal of Dairy Science* **99**, 5916-5925.

Rumball W, Keogh R, Miller J, Claydon R (2003) 'Choice'forage chicory (Cichorium intybus L.). *New Zealand Journal of Agricultural Research* **46**, 49-51.

Ryden J, Ball P, Garwood E (1984) Nitrate leaching from grassland. *Nature* **311**, 50.

Sanderson M, Wedin W (1989) Nitrogen in the detergent fibre fractions of temperate legumes and grasses. *Grass and Forage Science* **44**, 159-168.

Sanderson MA, Labreveux M, Hall MH, Elwinger GF (2003) Nutritive value of chicory and English plantain forage. *Crop Science* **43**, 1797-1804.

Schwinning S, Parsons AJ (1996) Analysis of the coexistence mechanisms for grasses and legumes in grazing systems. *Journal of Ecology* **84**, 799-813.

Shepherd M, Lucci G (2013) A review of the effect of autumn nitrogen fertiliser on pasture nitrogen concentration and an assessment of the potential effects on nitrate leaching risk. In 'Proceedings of the New Zealand Grassland Association' pp. 197-201.

Silsbury J (1970) Leaf growth in pasture grasses. *Tropical Grasslands* **4**, 17-36.

Sinclair T, Horie T (1989) Leaf nitrogen, photosynthesis, and crop radiation use efficiency: a review. *Crop Science* **29**, 90-98.

Skinner RH, Gustine DL, Sanderson MA (2004) Growth, water relations, and nutritive value of pasture species mixtures under moisture stress. *Crop Science* **44**, 1361-1369.

Smith GS, Cornforth IS, Henderson HV (1985) Critical leaf concentration for deficiencies of nitrogen, potassium, phosphorus, sulphur and magnesium. *New Phytologist* **101**, 393-409.

Sparling G, Schipper L (2002) Soil quality at a national scale in New Zealand. *Journal of Environmental quality* **31**, 1848-1857.

Statistics New Zealand (2017) Global New Zealand – International trade, investment, and travel profile: Year ended December 2016. Wellington: Ministry of Foreign Affairs and Trade, and Stats NZ. Available from www.stats.govt.nz

Stewart AV (1996) Plantain (Plantago lanceolata) – a potential pasture species. *Proceedings of the New Zealand Grassland Association* **58**, 7786.

Stout WL, Schnabel RR, Priddy WE, Elwinger GF, Fales SA, Muller LD (1997) Nitrate Leaching from Cattle Urine and Feces in Northeast USA. *Soil Science Society of America Journal* **61**, 1787-1794.

Tamminga S (1992) Nutrition Management of Dairy Cows as a Contribution to Pollution Control. *Journal of Dairy Science* **75**, 345-357.

Tamura Y, Nishibe S (2002) Changes in the concentrations of bioactive compounds in plantain leaves. *Journal of Agricultural and Food chemistry* **50**, 2514-2518.

Thomson KF, Poppi DP (1990) Livestock production from Pasture. In 'Pastures: Their Ecology and Management'. (Ed. RHM Langer) pp. 263-283. (Oxford University Press: Oxford).

Totty VK, Greenwood SL, Bryant RH, Edwards GR (2013) Nitrogen partitioning and milk production of dairy cows grazing simple and diverse pastures. *Journal of Dairy Science* **96**, 141-149.

Turner LR, Donaghy DJ, Lane PA, Rawnsley RP (2006) Effect of defoliation management, based on leaf stage, on perennial ryegrass (Lolium perenne L.), prairie grass (Bromus willdenowii Kunth.) and cocksfoot (Dactylis glomerata L.) under dryland conditions. 1. Regrowth, tillering and water-soluble carbohydrate concentration. *Grass and Forage Science* **61**, 164-174.

Valentine I, Kemp PD (1999) Chapter 19: Sustainability of Pastures and Crops. In 'New Zealand Pasture and Crop Science'. (Eds J White, J Hodgson) pp. 293-305. (Oxford University Press: Auckland).

Van Loo E (1992) Tillering, leaf expansion and growth of plants of two cultivars of perennial ryegrass grown using hydroponics at two water potentials. *Annals of Botany* **70**, 511-518.

Van Rossum MH, Bryant RH, Edwards GR (2013) Response of simple grass-white clover and multi-species pastures to gibberellic acid or nitrogen fertiliser in autumn. *Proceedings of the New Zealand Grassland Association* **75**, 145-150.

Van Soest PJ (1994) Nutritional Ecology of the Ruminant (2nd edn.) p. 476. (Cornell University Press: Ithaca, NY).

Vartha E (1977) Comparative growth of 'Grasslands Matua'prairie grass,'S23'ryegrass, an experimental cocksfoot, and 'Grasslands Kahu'timothy at Lincoln, Canterbury. *New Zealand journal of Experimental Agriculture* **5**, 137-141.

Volaire F, Lelievre F (2001) Drought survival in Dactylis glomerata and Festuca arundinacea under similar rooting conditions in tubes. *Plant and Soil* **229**, 225-234.

Wachendorf M, Büchter M, Trott H, Taube F (2004) Performance and environmental effects of forage production on sandy soils. II. Impact of defoliation system and nitrogen input on nitrate leaching losses. *Grass and Forage Science* **59**, 56-68.

Waghorn G, Clark D (2004) Feeding value of pastures for ruminants. *New Zealand Veterinary Journal* **52**, 320-331.

Waite R (1958) The water-soluble carbohydrates of grasses. IV. The effect of different levels of fertilizer treatment. *Journal of the Science of Food and Agriculture* **9**, 39-43.

Watkinson JH, Kear MJ (1994) High performance ion chromatography measurement of sulfate in 20 mM phosphate extracts of soil. *Communications in Soil Science and Plant Analysis* **25**, 1015-1033.

White J, Millner J, Moot D (2007) Cereals In 'New Zealand Pasture and Crop Science'. (Eds J White, J Hodgson) pp. 213-234. (Oxford University Press: Auckland).

Whitehead DC (2000) Uptake Concentrations of Nutruent Elements in Grassland Herbage. In 'Nutrient Elements in Grassland. Soil - Plant - Animal Relationships' pp. 41-69. (CAB International: Wallingford, UK).

Wilkins PW, Allen DK, Mytton LR (2000) Differences in the nitrogen use efficiency of perennial ryegrass varieties under simulated rotational grazing and their effects on nitrogen recovery and herbage nitrogen content. . *Grass and Forage Science* **55**, 69-76.

Williams R (1932) Effect of a nitrogenous manure on white clover and a comparison of the productiveness of four types of white clover under simulated pasture conditions. *Welsh Journal of Agriculture* **8**, 163.

Williams R (1955) Redistribution of mineral elements during development. *Annual Review of Plant Physiology* **6**, 25-42.

Wilman D (1975) Nitrogen and Italian ryegrass: 1. Growth upto 14 weeks: dry matter yield and digestibility *Grass and Forage Science* **30**, 141-147.

Woledge J (1988) Competition between grass and clover in spring as affected by nitrogen fertiliser. *Annals of Applied Biology* **112**, 175-186.

Woods R, Cameron K, Edwards G, Di H, Clough T (2016) Effects of forage type and gibberellic acid on nitrate leaching losses. *Soil Use and Management* **32**, 565-572.

Woodward S, Waugh C, Roach C, Fynn D, Phillips J (2013) Are diverse species mixtures better pastures for dairy farming. *Proceedings of the New Zealand Grassland Association* **75**, 79-84.

Woodward SL, Waghorn GC, Bryant MA, Benton A (2012) Can diverse pasture mixtures reduce nitrogen losses. *Proceedings of the 5th Australasian Dairy Science Symposium* **5**, 13-15.

Young DJ (1958) A study of the influence of nitrogen on the root weight and nodulation of white clover in a mixed sward. *Grass and Forage Science* **13**, 106-114.